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VOL. XLVII

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THE HAWAIIAN PLANTERS' RECORD

VOL. XLVII

H. L. LYON, *Editor*

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C. E. PEMBERTON

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W. L. McCLEERY

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ORGAN OF THE EXPERIMENT STATION OF THE
HAWAIIAN SUGAR PLANTERS' ASSOCIATION

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1943

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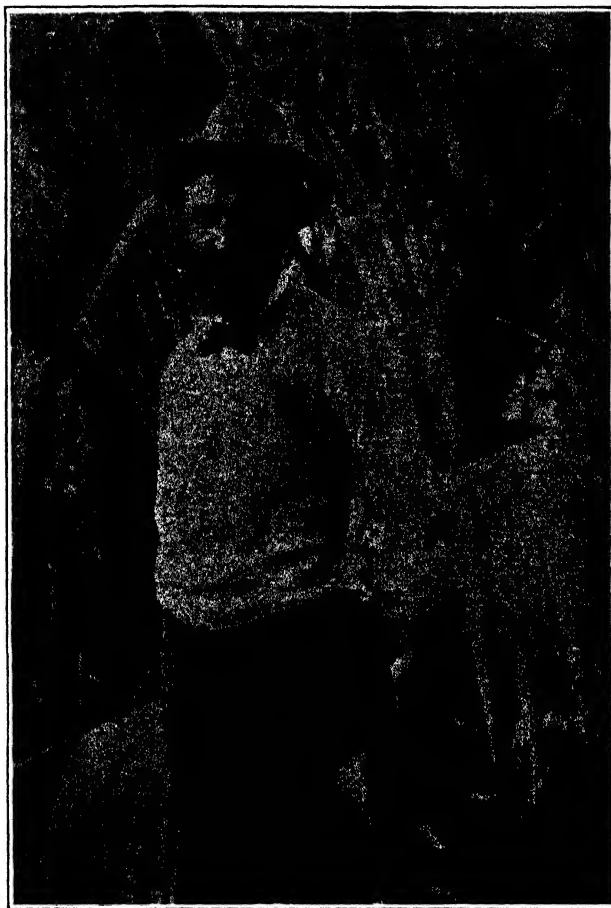
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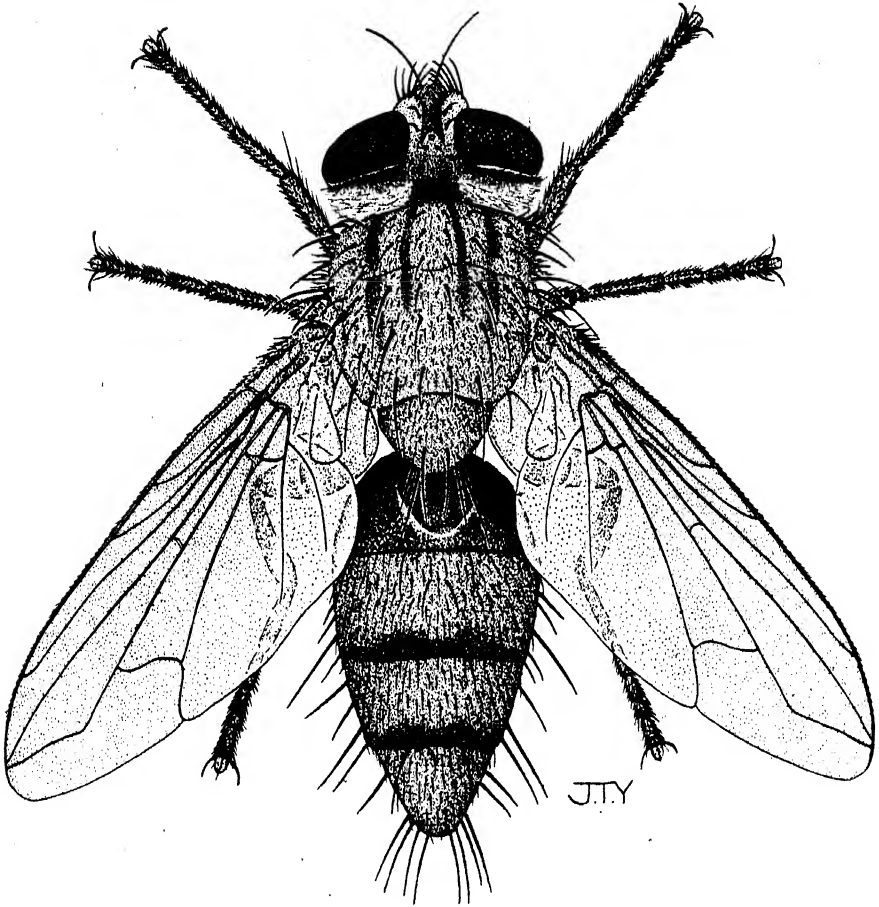
ILLUSTRATIONS APPEARING ON THE COVERS OF
VOLUME XLVII

FIRST QUARTER



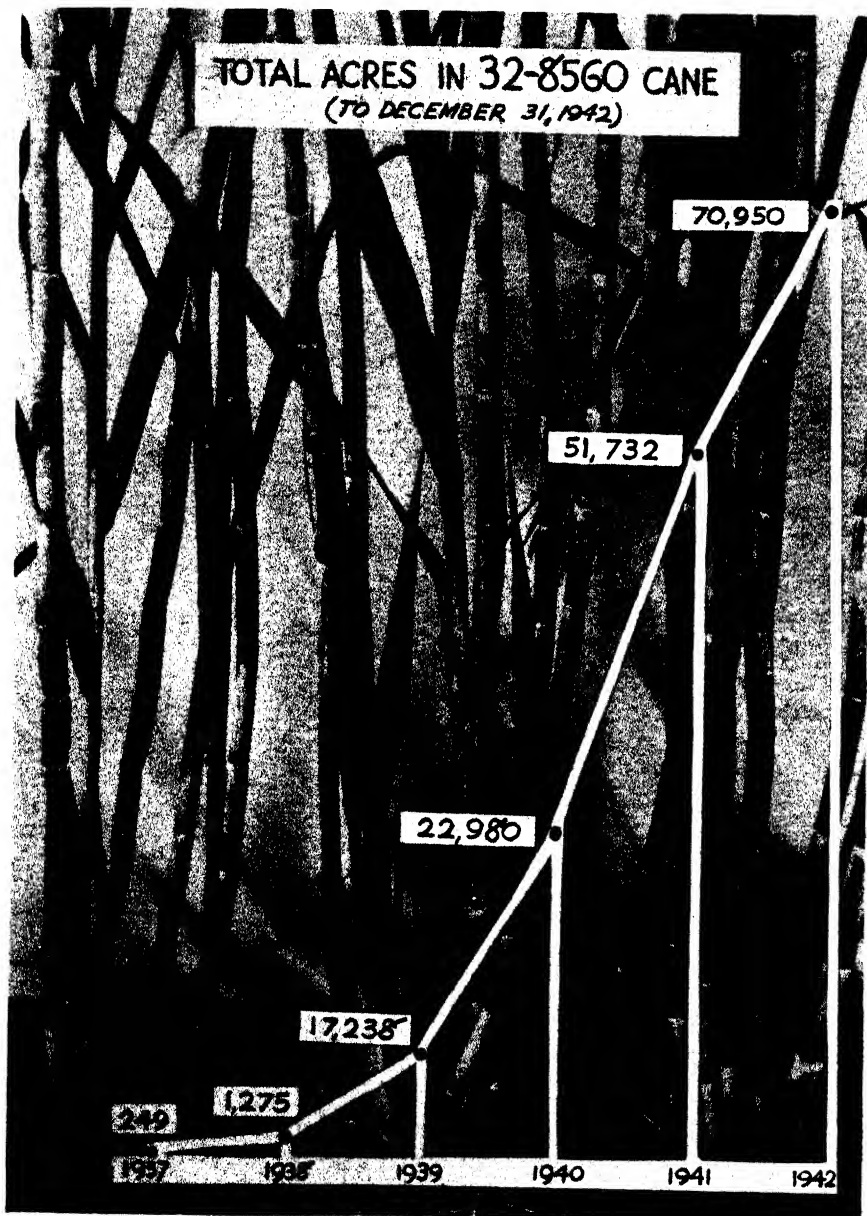
Albert Koebele, a pioneer in the biological control of insects, photographed in a Honolulu garden about 1900. He was the first entomologist to introduce beneficial insects into Hawaii, many of which have been and continue to be of great value to the Territory. His work commenced in 1895 and was carried on for many years. His extensive collection of useful lady-beetles has finally been put in order by P. H. Timberlake, who treats with part of this collection in the present issue.

SECOND QUARTER



Eucelatoria armigera (magnified about 12 times), a beneficial tachinid fly parasitic in a variety of destructive caterpillars. Its attempted introduction here from Mexico in 1923 was unsuccessful, in the absence of air transport at the time. Now, some twenty years later, unaided, it has succeeded in reaching these Islands, probably within worm-infested tomatoes from the mainland. In the field it attacks both the corn-ear worm and the green garden looper; it is hoped it will prove effective against the nutgrass armyworm also, in which it breeds readily in the laboratory. Its life cycle, a matter of some three weeks even during the cooler months of the year, is unusually brief for an insect of this kind. During an adult life of two weeks or longer it produces 40 or more offspring. Although slightly larger than *Ceromasia*, it is liable to be confused by the casual observer with that fly enemy of the sugar cane beetle borer. The female lays maggots which are inserted within its caterpillar victim by means of a highly specialized, thorn-like larvipositor.

THIRD QUARTER



FOURTH QUARTER



THE HAWAIIAN PLANTERS' RECORD

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No. 1

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The Coccinellidae or Ladybeetles of the Koebele Collection—Part I

By P. H. TIMBERLAKE

During his career in Hawaii engaged in introducing beneficial insects, Albert Koebele accumulated a considerable collection of Coccinellidae, or ladybeetles, from many parts of the world, particularly California, Mexico, Australia, China, and Japan. After his death, the widow of Mr. Koebele presented this large collection of many thousand specimens to W. M. Giffard who, in turn, on October 12, 1927, assigned the collection to the Experiment Station, H.S.P.A. Much of the material was unnamed and as its value for reference depended on its being properly classified, named, and arranged, it was desirable that this should be done. Fortunately it was possible to arrange with P. H. Timberlake to undertake this task while on sabbatical leave from the Citrus Experiment Station, Riverside, California in 1930-31. He was particularly qualified for this task, having worked considerably with Coccinellidae while on the staff of the Experiment Station, H.S.P.A., prior to 1924. It proved to be too much of an undertaking for the time available. However, a large portion was completed, and it seems that it is desirable to publish it as Part I, leaving the remainder, when finished, for some future publication.

The present paper is published as a memorial in recognition of the valuable services of Mr. Koebele in the biological control of many of the troublesome insect pests in Hawaii.

It seems fitting to reprint here the accounts of Mr. Koebele's work in Hawaii, as given by Dr. R. C. L. Perkins and O. H. Swezey, on pages 359-368 of The Hawaiian Planters' Record, Vol. XXIX, 1925.

OTTO H. SWEZEY.

THE EARLY WORK OF ALBERT KOEBELE IN HAWAII

By R. C. L. PERKINS

It was in 1890 that Koebele sent *Novius cardinalis* to Honolulu from California, where he had previously established it. Its success in controlling the cottony cushion scale, then one of the worst insect pests in the United States, had come to the notice of Mr. Jaeger, of Honolulu, who was interested in agriculture and horticulture, and it was through his sagacity that specimens were obtained from Koebele and introduced into Hawaii. As every one who pays any attention to such matters is aware, at this time, and previously, the above-mentioned pest was fully as destructive in the Islands as in California (where, of course, the interests were much greater) and some other "blights," as these insect pests were frequently termed, were almost equally destructive.

Thus we read that coffee, which had been introduced in 1825, by the middle of the century had become quite an industry on Kauai, but this was abandoned in 1856 owing to the ravages of "blight" said to have been introduced in 1850.

In 1876, the Rev. T. Blackburn, an expert entomologist in Honolulu, wrote that "the fruit trees were afflicted with an incurable blight."

The success of the *Novius*, which soon became manifest, led to Koebele's subsequent engagement partly by the Hawaiian Sugar Planters' Association and partly by the Government, as was urged by Mr. Jaeger.

Early in 1892, the writer became acquainted with Mr. Jaeger, who was naturally very enthusiastic, not only about what had already been achieved, but as to what further success Koebele might attain. He was very much surprised when informed that the latter's work as an entomologist was little, if at all, known except in the United States.

In 1894, many Coccinellidae were introduced by Koebele, chief among which were *Cryptolaemus montrouzieri* and *Coelophora inaequalis*. In the earlier literature the latter is unfortunately always referred to as "*Coccinella repanda*," the name used by Koebele, and it was not till many years afterwards that we received named specimens of the true *repanda* from Australia and became aware of this error.

Probably either of these two ladybirds was even more valuable to the Islands than the *Novius*, owing to the fact that they preyed on pests which seriously attacked a larger number of plants than did the cottony cushion scale.

In 1892, throughout the Kona district, where I was then stationed, *Pulvinaria* covered many of the trees, which were in a dying condition and many, in fact, were dead. After the *Cryptolaemus* was taken there, this scale to a large extent disappeared. There may be some who still remember the strange appearance of the trunks of many of the larger ornamental trees in Honolulu in June, 1896, when the *Cryptolaemus* larvae, having become full-grown, congregated together so as to form large white patches covering several square feet of the surface, which was entirely hidden by them. Owing to the white covering of the larvae many people mistook these for scale insects and were actually destroying them. Photographs were taken of some of these tree trunks and copies are probably still extant in the possession of the Territorial Board of Agriculture and Forestry.

In June, 1895, many of the native trees in the kipukas that are found in the forest at various parts within eight or ten miles of the crater of Kilauea were covered with a conspicuous black Aphid and others less noticeable. Many of the *Pelea* trees, especially, were in a dead or dying condition, but other forest trees were affected. At this time single specimens of *Coelophora* were rarely noticed, having evidently just arrived at the locality. In September, the ladybirds were in thousands and when the same places were revisited in August, 1896, we were unable to find even a single specimen of the large black Aphid.

Other ladybirds introduced at this early period were *Rhizobius ventralis*, *Platynus lividigaster* and *Orcus chalybeus*, all of which were more or less successful and useful. Others were fully established at large, but later became either scarce or totally extinct. The history of some of these is interesting. No species appeared more promising for a time than *Chilocorus circumdatus*. At first the larvae became extraordinarily numerous, entirely clearing some trees

of the harder scales, but gradually the beetle became rarer and rarer, till after 1900 I myself saw only a few single individuals. *Novius koebelei*, so far as we are aware, disappeared still earlier, and so far as we know *Synonyche grandis*, by far the largest of introduced ladybirds, was common for only a very short time on some of the ornamental bamboos in Honolulu. *Leis conformis*, from which much was expected, was almost entirely a failure. The pretty *Coccinella pupillata* was noticed from time to time, but not in great numbers.

During the earlier years of his work, Koebele's visits to Honolulu were short and few, his material being liberated by the Commissioner of Agriculture, Joseph Marsden. Whatever may have been the latter's knowledge of agriculture, of entomology he had none beyond that of a few Latin names, to repeat which gave him a good deal of pleasure. No doubt Koebele's chief success with ladybirds as compared with other insects was due to this fact, for when liberated, full instructions having been given as to the place where they were to be turned out, they could look after themselves. But many small parasites were also sent, and of almost none of these is there any record, except *Chalcis obscurata*, which became very abundant throughout the Islands. As Koebele informed me, his special request that all the dead material should be saved for subsequent identification, was not attended to in any instance.

Owing to my own occupation in the forests of the different Islands and to Koebele's infrequent visits to Honolulu, we did not happen to meet during the earliest years of his work, but on returning from a considerable stay in the Kauai forests in 1895, I found him in Honolulu. Among other matters, we discussed the possibility of his economic introductions proving antagonistic to my own work on the native fauna, especially as some of the rarer species of native Hemerobiids, which had been unusually numerous on the Aphis-infested *Pelca* trees at Kilauea, had disappeared after the introduced *Coelophora* had eaten up the Aphis. Reference was made to this point in a brief account of Koebele's work which I wrote in 1896 and which was published in the following year in *Nature*. Imperfect as this account was, it at least had the effect of calling the attention of most European countries to his work and its possibilities.

As it was necessary for me, after I had met him, to do further work in East Maui, I persuaded Koebele to accompany me and share my tent, since he was anxious to obtain some knowledge of the forest insects, concerning which there were complaints. After spending some weeks in the high, wet forest of the windward side, we left our tent, and carrying as few *impedimenta* as possible, we worked about the summit and through the crater to the lee side, sleeping in the open or in such natural shelters as were available. Being lightly clothed we were a good deal troubled by the sharp frosts at night, which appeared abnormally cold after the fine hot weather of the daytime. I have referred to this, the first of several hard trips we made together, as it gave me the first opportunity to see what a very accomplished field worker Koebele had become. He was particularly expert at collecting difficult beetles, and had no doubt learned many wrinkles from his friend E. A. Schwarz, of Washington, in earlier years.

In 1897, I joined Koebele in California and accompanied him to Arizona and Mexico, where he hoped to obtain some natural enemy for the mealybug of the alligator pear. This had now become an unsightly pest, having been introduced since the earlier years of my collecting. The complaints about Lantana were already numerous and some preliminary investigation was made of the plant in Mexico, but at this time no attempt was made to introduce any of the insects attacking it.

In 1898 and 1899, I was in England working at the *Fauna Hawaliensis*, but on my return (early in 1900) to the Islands I was still more in contact with Koebele than previously, since we not only made many collecting trips in company, but I did much study work in his office.

In 1900, the presence of a number of insects entirely unknown to me in 1897 was obvious. Chief among these was the melon fly (*Dacus cucurbitae*) and the cane leafhopper. The latter was noted first as a leafhopper new to the Islands, at Waialua, where it occurred in some numbers around the electric lights in 1900. Its connection with sugar cane was not known at the time; in fact not until a year or two afterwards, when it was reported by August Ahrens as injuring the cane on Oahu Plantation. There is no doubt that as a pest it first showed up on that plantation and it was probably introduced, or at least became established there first, about the year 1897. Had this insect occurred in earlier years, it is unlikely (considering its attraction by light) that I should not have noticed it, and quite impossible that Koebele, who was perfectly well acquainted with the accounts of the Javanese *vastatrix*, and was frequently in the cane fields investigating the cane borer and other cane insects, should have overlooked

it. In fact when he first saw the leafhopper in the Islands he took it to be the same as the pest recorded from Java.

Also in 1900, the fern weevil (*Syagrius*) was first noticed in fernhouses in Honolulu on maiden-hair ferns only, and the possibility of its spreading to the native tree ferns of the forest was not thought of.

The introduction of the melon fly must have taken place at about the same time. Up to the time when I left the Islands in 1897 melons were almost a drug on the market, and except possibly for the Chinese, could hardly have paid for raising. After my return, one of the first settlers in the agricultural colony at Wahiawa informed me that he considered the presence of the melon fly by no means a calamity, as he was able to raise melons by adopting certain precautions and obtain a good price for them, whereas formerly it would not pay to grow them. The species was first described in 1899 from specimens obtained by Mr. Compere in Honolulu. Koebele had, however, previously collected the species on one of his trips to the Orient, before it was known in the Islands, and had noted it as a pest on plants of the melon family. In 1900, I found Hawaiian melons and cucumbers were almost unprocurable. It does not appear that Koebele made any concentrated effort to obtain parasites for *Dacus*, possibly because Compere was specially investigating fruit flies at the time. In Mexico, when engaged on Lantana work, he noted a fine parasite on other fruit flies, but he would not take the risk of attempting to send over puparia of these flies, for fear that by some accident the flies themselves might be introduced. He was always cautious in his introductions and many of the Australian ladybirds he was afraid to send in their larval state for fear of introducing their parasites.

In addition to the one species, *Chalcis obscurata*, above mentioned, Koebele sent over many other parasitic Hymenoptera from various countries, Australia, America and the Orient, at a time when he was the only economic entomologist connected with the Islands. Many of these were parasites of scale insects and *Aleyrodes*, but *Ichneumonidae* were sent from America and *Braconidae* from Oriental countries.

No record exists of the minute scale parasites which were frequently sent. For instance, it is quite uncertain whether some of the established parasites on scales were the results of his sendings or whether they came by other means, but it is hardly probable that of the large number of species sent none survived, even though they had no expert handling on arrival. As Koebele was well acquainted with the exact localities of the scale insects in various grounds and gardens in Honolulu and gave exact directions, as to where the parasites were to be liberated, a certain amount of success probably resulted. Although he never cared to make any attempt to introduce birds, various species of frogs, toads and bats were sent, but the latter at any rate failed to become established, though individuals were seen alive for a year after their introduction.

Of his later work on the Lantana plant, the sugar cane leafhopper and some other less important insect pests, fuller published records exist and can be consulted.*

Koebele was *par excellence* a field worker in entomology and his knowledge of living insects was of a most extensive character, as at one time or another he paid special attention to all orders, but chiefly to Coleoptera and Lepidoptera, to some of the minute Hymenoptera and to scale insects. At one period he did much rearing of micro-Lepidoptera for Professor Riley. As may be judged from the nature of his field work, the Coccinellidae or ladybirds were his especial favorites, and he collected great numbers of species in the various countries he visited. He was not a great reader of entomological literature, but certain systematic works he used continually, e.g., Maskell's and Green's Coccidae, and especially Crotch's book on the Coccinellidae, which accompanied him on all his travels. Of the classification and specific character structures of these groups he had an extensive knowledge, though he published no notes of a systematic nature on others excepting some official reports and even these were to him an uncongenial task.

* The Introduction into Hawaii of Insects that Attack Lantana by R. C. L. Perkins and O. H. Swezey. Entomological Series Bull. No. 16, Experiment Station, H.S.P.A., 1924.

Biological Control of the Sugar Cane Leafhopper in Hawaii by O. H. Swezey. Entomological Series Bull. No. 21, Experiment Station, H.S.P.A., 1936.

His success in the field was due to his acute perception of the habits of insects, and unsurpassed perseverance, and he was naturally a very quick worker, so that with insects that are rare and difficult to obtain he could collect a greater number in a given time than most of the best field workers we have known. Under any circumstances he was a most pleasant companion on a trip, for even when the hardest and most uncomfortable conditions were added to ill success he remained cheerful and good humored, hoping to the last to achieve something by which a failure might be converted into a triumph. He met with many adventures in his varied traveling, and in unhealthy countries contracted many fevers, which failed to lessen his enthusiasm for his work, but he rarely spoke of his adventures. In his younger days, when collecting in Florida, he was down with severe fever and has told us how, at the time, numbers of a fine Sphingid moth, the caterpillars of which he had laboriously collected, were emerging in numbers in the room in which he lay, and how he spent the night alternately in killing the specimens, lest they should damage themselves, and in lying in a fainting condition on the floor. As would naturally be expected, he was the discoverer of great numbers of species of insects which were new to science, and many were named after him by their describers.

BIOGRAPHICAL SKETCH OF THE WORK OF ALBERT KOEBELE IN HAWAII

By O. H. SWEZEY

Mr. Albert Koebele was the pioneer economic entomologist in the Hawaiian Islands. He was one of the first, if not the very first, entomologist to engage in the introduction of their natural enemies as a method of combating insect pests. His early work in this line was in California, where he introduced from Australia in 1888-89, the lady beetle *Novius cardinalis* Muls. as an enemy to the cottony cushion scale, *Icerya purchasi* Mask., a serious citrus pest. This was a remarkable success, and was considered to have saved the citrus industry from ruin.

At this time, Koebele was in the employ of the U. S. Department of Agriculture, an appointment which commenced in 1881-82. It was in 1885 that he was transferred to the Pacific Coast region, where he established his home at Alameda, California. During the several years that he was working in California he was chiefly engaged in the introduction of beneficial insects. Two trips were made to Australia for this purpose. This period of work ended on September 30, 1893, when he resigned from the U. S. Department of Agriculture to take up similar work in Hawaii, at first under a Commissioner of Agriculture of the provisional government, later as entomologist of the Board of Agriculture and Forestry, after the latter was organized, and about 1903 or 1904 was placed on the staff of the Experiment Station, H.S.P.A., as consulting entomologist, which position he held at the beginning of the Great War.

It has been impossible to obtain exact records of the work and travels of Mr. Koebele during his early work in Hawaii. However, during 1894-95, he made an extensive tour of Australia, Ceylon, China and Japan in search of beneficial insects desirable of introduction. Many lady beetles were introduced at this time. Among those which have become established and continue effective up to the present are *Cryptolaemus montrouzieri* Muls., *Rhizobius ventralis* Erich. from Australia preying on mealybugs; *Orcus chalybaeus* (Boisd.), *Serangium maculigerum* Blkb. from Australia, *Chilocorus circumdatus* Schon. from China, *Sticholotis punctatus* Crotch from China, all on diaspine scale insects; *Coclophora inaequalis* (Fab.), *Platyomus lividigaster* Muls., *Diomus notescens* (Blkb.) from Australia and *Coclophora pupillata* (Schon.) from Hongkong, all preying on plant lice. Among parasites that were introduced on this trip are *Chalcis obscurata* Walker and *Microbracon omiodivorum* (Terry), both from Japan and parasitic on leafroller caterpillars, the *Microbracon* being especially effective on the sugar cane leafroller.

In 1896-97 considerable time was spent in Mexico, Arizona and California, from which places large quantities of lady beetles were sent, also many kinds of cutworm enemies, but apparently these mostly failed to become established.

In 1899-1900, Koebele went on another trip to Australia, spending some time in Fiji on

the way, and also going to Hongkong on the return voyage. Many shipments of beneficial insects were made on this trip, particularly from Australia.

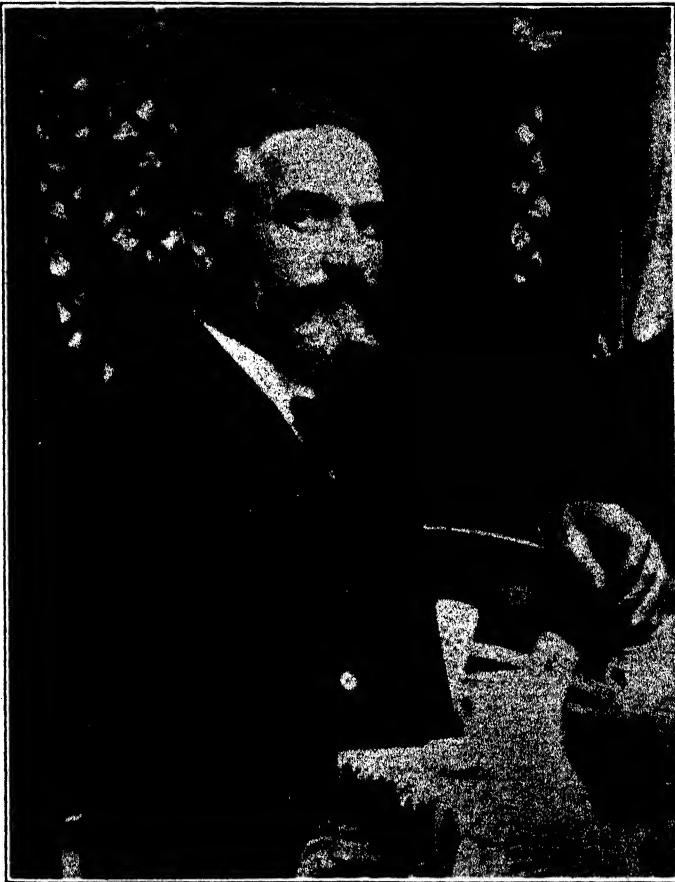
During the greater part of 1902, Koebele was studying the insects affecting lantana in Mexico, and sending to Honolulu those which he found to be particularly attached to lantana and not likely to become injurious to any other plants. At that time, Dr. R. C. L. Perkins was employed by the Territory and assisted Koebele in his work. The parasite material sent by Koebele was taken care of by Perkins and liberations made in favorable places for them to become established, at the same time destroying parasites, many of which affected most of the insects which it was desired to introduce. The enemies to lantana which were successfully introduced were as follows: Two butterflies, *Thecla echion* Linn. and *Thecla agra* Hew., whose larvae feed on lantana flowers; two moths, *Crociosema lantanæ* Busck, and *Platyptilia pusillidactyla* Walker, whose larvae destroy the lantana flower clusters; a leaf-miner, *Cremastobombycia lantanella* Busck, whose larvae feed inside the leaves; a leaf-bug, *Teleonemia lantanæ* Dist., the young of which feed so numerously on the under side of the leaves as to destroy them and check the new growth of the plant sufficiently as to prevent flowering; a stem gall-fly, *Eutreta xanthochaeta* Ald., whose larvae live in enlargements of the freshly growing stems; a seed-fly, *Agromyza lantanæ* Frogg., whose larvae feed in the fruits, often destroying the seeds, and usually causing the fruits to dry up, so that they are not eaten by birds with the resultant scattering of seeds. The combined results of the work of these eight introduced insects is to greatly reduce the enormous production of seeds that formerly occurred on lantana and which were so widely dispersed by the ripened fruits being eaten by birds.

During the summer of 1903, Koebele investigated leafhopper parasites in Ohio, where the writer had published a note on a dryinid parasite of a leafhopper occurring in grass lands of that region. He sent many hundreds of these parasites, and other leafhopper parasites that he discovered, to Hawaii to be tried on the sugar cane leafhopper which at that time was becoming very destructive on many of the sugar plantations. All of these Ohio parasites failed, and in the early summer of 1904, with Perkins, Koebele went to Australia in search of leafhopper enemies. Many were discovered in Queensland and attempts made at their introduction. The only successful introductions were four egg-parasites, the most important of which was *Paranagrus optabilis* Perkins, the second in importance being *Ootetrastichus beatus* Perkins, which was secured in Fiji, where Koebele stopped a short time on the return from this trip. The work of these egg-parasites resulted in greatly checking the leafhopper pest so that it was no longer a menace to the sugar industry. Another introduction at this time was *Aphanomerus pusillus* Perk., an egg-parasite on the torpedo-bug *Siphanta acuta* Walker, which was a pest on coffee, citrus and other garden and ornamental trees.

When he returned from Fiji, a short time was spent in Honolulu in the summer of 1905, which was the last time Koebele was in Honolulu. He went to California shortly afterward, and at different times in 1906-8 attention was given to the study of sugar cane insects and their parasites in Mexico and to the enemies of hornfly in Mexico and Arizona. A number of minor introductions were made. Those that succeeded were: *Eucoila impatiens* Say from Arizona in 1906, a parasite on dipterous larvae in cow dung; *Azya luteipes* Muls. from Mexico in 1908, a lady beetle feeding on Lecanium scales; *Hyperaspis jocosa* (Muls.), from Mexico in 1908, a lady beetle feeding on *Orthezia insignis* Douglas.

The latter part of 1908, Koebele went to Waldkirch, Germany, his boyhood home, where he was born in 1852. This was mainly as an opportunity for the recovery of his health which had been greatly impaired by so much time spent in entomological exploration and research work in fever-infested regions of the tropics. While there during the summers of 1909-11, he studied the enemies of hornfly, and sent much material to Honolulu, but little, if any, success was obtained by this. In 1910, on account of continued failing health, he was relieved from active duty, though still retained as Consulting Entomologist by the H.S.P.A. He continued living in Germany and was there during the Great War, on account of which he was reduced to very meager circumstances and both he and his wife suffered great hardships. At the close of the war, as soon as it was learned of their circumstances, attempts were made by the Hawaiian Sugar Planters' Association to arrange for their return to their home in Alameda, California. By the time that all arrangements were completed, however, he had become too feeble for undertaking such a trip. He continued to fail and his death finally occurred December 28, 1924, in his 73rd year.

The services rendered by Mr. Koebele and the benefits derived by the agricultural and horticultural interests of Hawaii by his introduction of beneficial insects cannot be estimated in dollars and cents. He made the beginning in this line of work, and much of the time was



ALBERT KOEBELE

Enlargement from a family group taken at his former home in Germany.
(Courtesy of W. M. Giffard.)

working alone, yet seventeen species of ladybeetles were successfully introduced by him and have become valuable factors in keeping reduced such pests as scale insects, mealybugs, plant lice and leaf-mites. At least six other ladybeetles were introduced and became established, but after a few years disappeared. The eight lantana insects were introduced by him, and about the same number of miscellaneous parasites of Diptera, Lepidoptera, etc. Following Koebele in this line of work, the other entomologists have introduced a larger number of beneficial insects and some of them have produced more valuable results, but this should not in any way lessen the credit to be given to him who was the pioneer in Hawaii in this important phase of entomological work.

Papers and reports by Koebele or concerning his Hawaiian work were published as follows:

Report of a trip to Australia to investigate the natural enemies of the fluted scale. U.S.D.A., Ent. Bul. 21, 1890.*

Studies of parasitic and predaceous insects in New Zealand, Australia and adjacent Islands.*
 U. S. Dept. of Agriculture, pp. 1-39, 1893.
 Professor Koebele and his work. *Planters' Monthly*, XV, p. 103, 1896.
 Report on insect pests. *Planters' Monthly*, XV, pp. 590-598, 1896.
 Report of the entomologist of the Hawaiian government. *Planters' Monthly*, XVI, pp. 65-85, 1897.
 Report of Professor Albert Koebele, entomologist of the Hawaiian government. *Planters' Monthly*, XVII, pp. 208-219, 258-269, 1898.
 Report of Professor Albert Koebele, entomologist. Report of the Com. of Agr. and Forestry for 1900, pp. 36-49, 1901. Also in *Planters' Monthly*, XX, pp. 299-309, 1901.
 Report of Professor Koebele on destruction of forest trees, Hawaii. Rept. of the Com. Agr. and Forestry, Hawaii, for 1900, pp. 50-60, 1901.
 Notes on insects affecting the Koa trees at Haiku forest on Maui. Rept. of the Com. Agr. and Forestry, Hawaii, for 1900, pp. 61-66, 1901.
 Report of Professor Koebele on *Lantana* scale. Rept. of the Com. Agr. and Forestry, Hawaii, for 1901-02, pp. 54-65, 1903.
 Report of Professor Albert Koebele. Third Report of the Board of Com. of Agr. and Forestry, Hawaii, for 1906, pp. 159-164, 1907.
 Insect investigations in Mexico. Fourth Rept. Board of Com. of Agr. and Forestry, Hawaii, pp. 89-97, 1908.
 Report on the enemies of *Lantana camara* in Mexico, and their introduction into the Hawaiian Islands. Ent. Bul. No. 16, Exp. Station, H.S.P.A., pp. 54-71, 1923.

PREFACE

The following paper is the first of a series of parts dealing with the Koebele collection of Coccinellidae, which it is hoped may be finally completed. The first part deals solely with the tribe Coccinellini, or subfamily Coccinellinae as I prefer to call it. The species found in the collection are listed in what is believed to be the most natural sequence, with descriptions of such varieties and species that appear to be new. The notes in brackets are copied verbatim from Mr. Koebele's field note books and will be found to add ecological data of great value in many cases. In the Appendix I have added tables and descriptions which could not be interpolated conveniently in the list.

It has been found advisable to introduce a considerable number of new genera, particularly in the forms allied to the old genera *Cycloneda* and *Coelophora*. It is with some misgivings that I propose these, for while they are strictly commensurate in value to those in use for the fauna of North America and Europe, there is some doubt that the fauna of the whole world will stand such fine division. Particularly is this true of the fauna of the tropics, both of the Old and the New World, where the species of Coccinellidae are more numerous than in temperate regions. It has been suspected by some workers that the genera of the tropics would be found to be much less stable, or with more annectent forms, than is true of those inhabiting the temperate regions, and this view is probably based more or less on fact. However, the genera here proposed are natural groups and distinct in the usual morphological as well as genital characters.

It is necessary to add that the new genera, that are founded on described and presumably correctly recognized species, are nevertheless based on my recognition

* This paper is concerned with Koebele's work before coming to Hawaii, but the knowledge gained thus was of great assistance when he began here, and deals with the same species as many of his early introductions to Hawaii.

of those species. This statement is made in view of Opinion 65 of the International Commission on Zoological Nomenclature, which seems to be interpreted by some authors as an excuse to accept all genotype designations at face value, even when it is known that certain species so designated are misdeterminations. It should be the aim of the taxonomist to apply names (both specific and generic) as close to the original usage as his knowledge permits. Any procedure which contraverts this principle seems to me decidedly unscientific.

P. H. TIMBERLAKE.

Riverside, Calif.
March 21, 1938

COCCINELLINAE

NAEMIA MULSANT

Naemia seriata (Melsheimer)

New Jersey, 2 specimens; Texas, 1; Staten Island, New York (Ormande), 1; Alameda, California (Koebele), 1.

PARANAEMIA CASEY

Paranaemia vittigera (Mulsant)

Siskiyou County, California, July (Koebele, No. 3037), 2; Alameda, California (Koebele), 2; Arizona (Koebele, No. 2429), 1; Guadalupe, Federal District, Mexico, Nov. 1907 (Koebele), 2.

I can not agree with Casey in the separation of *P. similis* Casey, and hardly believe that it is worthy of varietal or subspecific rank.

COLEOMEGILLA TIMBERLAKE

The generic name *Coleomegilla* was first used by me in Proc. U. S. Nat. Mus. 56, p. 139, unfortunately without credit to Cockerell, from whom the name had been received. Although I tried to correct this error later, I think it is necessary to take the literature as it stands and credit the name to the first user.

Leng has sought to synonymize *Coleomegilla* with *Ceratomegilla* Crotch, but I have examined the type species of the latter genus and believe that it is much more closely related to *Hippodamia* and *Adonia* than to *Coleomegilla*.

Coleomegilla maculata lengi new subspecies (*fuscilabris* Casey, Leng and other authors, but not Mulsant)

Rochester, New York, May 17, 1898, 4 (paratypes); Columbus, Ohio, Aug. 1903 (Koebele), 6 (holotype and paratypes).

This is the familiar *Coleomegilla* of eastern United States described and figured many times under the name of *maculata* or *fuscilabris* and hence requires no further description. When Leng described his *floridana* he evidently neglected to check the original description of *fuscilabris* (Mulsant) for otherwise he would have discovered that *floridana* is identical with *fuscilabris* which was described from New Orleans.

The race *decepta* (Blatchley) described as a form of *maculata* is not that species at all but is a southern race of *Naemia seriata* (Melsheimer). I have seen it from Beaufort, South Carolina, several localities in Florida and from Cuba and Haiti. It should be cited as *Naemia seriata decepta* (Blatchley).

Coleomegilla maculata strenua (Casey)

Sonora, Mexico (Koebele, No. 1680), 3.

("1680. Four specimens mounted from Hermosillo, Mex., April 1897, found in bush infested with aphids.")*

This is hardly distinct from the more northern and eastern *lengi* by the characters cited by Casey, although it does undoubtedly average a little larger in size. However, there is a slight difference in the male genitalia, hence I am disposed to recognize it as a subspecies.

Coleomegilla maculata medialis (Casey)

Cuautla, Morelos, Mexico, Nov. 21, 1907 (Koebele), 6.

This race of Central America and the more tropical parts of Mexico has the head more strongly punctured than in *strenua*, and the markings are somewhat different. It intergrades northward with *strenua* and I have seen annectent specimens from Rosario, Sinaloa; Tuxpan; and Paso del Norte, Chihuahua.

ERIOPSIS Mulsant**Eriopsis opposita** (Guérin)

Chile, 1 specimen.

ANISOSTICTA Chevrolat**Anisosticta 19-punctata** (Linnaeus)

Berlin, Germany (Weise), 1 male.

Anisosticta bitriangularis (Say)

Peekskill, New York, June 24, 1893 (Ormande), 1.

MACRONAEMIA Casey**Macronaemia episcopalis** (Kirby) (Plate I, Fig. 1)

Eldorado County, California, June, 1901 (Van Dyke), 1.

ADONIA Mulsant**Adonia variegata** (Goeze)

Waldkirch, Baden, Germany (Koebele), 3.

HIPPODAMIA Chevrolat**Hippodamia tibialis** (Say)

Columbus, Ohio (Koebele), 4; Kansas (T. B. Ashton), 2; Oregon (Koebele), 1.

Hippodamia parenthesis (Say)

Boston, Massachusetts, June 16, 1894 (Ormonde), 1; West Point, Nebraska, June, 1884, 1; Easton, Washington (Koebele), 1.

Hippodamia lunatomaculata (Motschulsky)

Oregon (Koebele, No. 11), 3.

Hippodamia apicalis (Casey)

Argus Mountains, California, May, 1891, on *Pinus monophylla* (Koebele), 1; Placer County, California (Koebele), 1, and 1 male without data.

* This and subsequent quotations are from Koebele's field note books.

Hippodamia sinuata Mulsant

San Francisco County, California (Horn), 1; Alameda, California (Koebele, No. 18), 3.

Hippodamia sinuata spuria Leconte

Oregon (Koebele, No. 12), 3; Alaska, 2.

Hippodamia koebelei Timberlake

Hippodamia convergens, var., Gorham, 1891, Biol. Centr. Amer. 7, p. 153, pl. 8, fig. 24 (at least in part).

Exactly resembling in size and coloration the immaculate variety of *Hippodamia convergens* and hitherto confused with it, but easily distinguished in the male sex by the dilated front and middle basitarsi. The female is hardly distinguishable except by the more or less uncertain character of having the pale border of the pronotum narrower and more even throughout.

[While this paper was awaiting publication, the description of *Hippodamia koebelei*, designated by Mr. Timberlake as a new species, was withdrawn and published in Proc. Ent. Soc. Washington, 44: 39, 1942. This was done as it was desired to use the name in other literature awaiting publication.—O. H. S.]

Mexico, May 22 and 27, 1922 (E. G. Smythe); 1 ♂ Mexico City (O. W. Barrett); 1 ♂ Oaxaca, Mexico (L. O. Howard); 1 ♂ Las Vegas, Mexico (Hoegel); 1 ♀ Mt. Diabola, Puebla, Mexico, July 29, 1901 (R. H. Hay); 1 ♀ Durango, Durango, Mexico (F. C. Bishopp); and 1 ♂, 1 ♀ Mexico (Koebele, No. 1687).

(See note No. 1687, under *H. convergens*.)

Types in U. S. National Museum, Cat. No. 55902, except the last female recorded above which belongs to the Koebele collection.

Hippodamia caseyi Johnson

Placer County, California (Koebele No. 8), 15 specimens; Easton, Washington (Koebele No. 9), 4.

This is the species called *lecontei* in my paper on *Hippodamia* (Jour. New York Ent. Soc., 27, p. 168 and 169, 1919). However, the real *lecontei* was described from New Mexico and, as I now recognize it, it is a form very close to *H. glacialis* and possibly only a subspecies. It occurs evidently along the eastern slope of the Rocky Mountains. The present species occurs from the Rocky Mountains westward to the Pacific Coast. It was found in the Casey collection under this name and as a part of the specimens were received from Johnson, the name is probably authentically applied, but the types of *caseyi*, if any are existent, should be investigated.

Hippodamia convergens Guérin

Newark, New Jersey, Aug. 16, 1896 (Ormonde), 1; San Francisco, California (Koebele, No. 5 and 6), 2; Placer County, California (Koebele), 8; Alameda, California (Koebele), 2; Santa Cruz Mountains, California, 1; Siskiyou County, California (Koebele, No. 7), 3; Eldorado County, California (Koebele), 2; Arizona (Koebele, No. 2417), 2; El Paso, Texas (Koebele, No. 1651), 1; Sonora, Mexico (Koebele, No. 1651), 4; Morelos, Mexico (Koebele, No. 1651), 5; Vera Cruz, Mexico (Koebele), 1; Mexico (Koebele, No. 1687), 10.

("1651. One specimen, El Paso, Texas; one at Cuautla, Morelos, Mexico; California. More from Cuautla.")

"1687. Common on pine and fir trees, base of Popocatepetl, Mexico, May 1897, and feeding upon aphids.")

Hippodamia 15-maculata Mulsant

West Point, Nebraska, June, 1888 (Bruner), 1 female.

Hippodamia moesta Leconte

Oregon (Koebele No. 23), 1 female.

Hippodamia moesta bowditchi Johnson

Alaska, 1 female.

From study of other material I have concluded that *bowditchi* is a form of *moesta*.

Hippodamia 5-signata uteana Casey

Goldfield, Nevada, June 27, 1907 (apparently collected by Nunenmacher), 6; Placer County, California (Koebele), 1; Siskiyou County, California, August (Koebele), 1; Panamint Mountains, California, April, 1891 (Koebele, No. 10), 6.

Hippodamia 5-signata ambigua Leconte

San Francisco County, California (Koebele, No. 2, 3 and 4), 6.

San Francisco is the type locality of *punctulata* Leconte, and *ambigua* was described from California and Oregon without a more definite type locality being indicated. After a careful consideration of the original descriptions I conclude that *ambigua* and *punctulata* are probably too similar to be kept separated satisfactorily, although I have seen little or no material from the northern part of California where typical *ambigua* should be found, if anywhere, in a more constant condition. In fact, typical *ambigua* as I deduce it from description is intermediate between *obliqua* Casey and *punctulata* Leconte. Specimens from southern California commonly have the pronotum white only on the anterior angles, but the posterior angles may have a smaller white spot, a white dash may occur at the middle of the anterior margin and two small white spots may occur on the disk. Specimens from the vicinity of San Francisco Bay commonly have the additional adornments and the pronotum also may be white clear across the anterior margin. Such is the condition as described for *punctulata*. *Ambigua* is described as having the lateral and anterior margins and two small discal spots white, with the discal spots and white anterior margin sometimes absent. It thus becomes a mere matter of choice or expediency whether to merge *obliqua* with *ambigua*, or to preserve *obliqua* and merge *punctulata* with *ambigua*. It is perhaps better to follow customary usage, unless all three names could be preserved, which might be a happy solution of the dilemma.

Hippodamia 5-signata obliqua Casey

Oregon (Koebele, No. 1), 4.

Hippodamia glacialis (Fabricius)

Columbus, Ohio, Aug. 1903 (Koebele), 3; Gravesend, Long Island, New York, Aug. 22, 1896 (Ormonde), 1.

Hippodamia extensa Mulsant

Alameda County, California (Koebele), 3.

SEMIADALIA CROTCH**Semiadalia notata** (Laicharting)

Waldkirch, Baden, Germany (Koebele), 8.

COCCINELLA LINNAEUS**Coccinella 7-punctata** Linnaeus

Waldkirch, Baden, Germany (Koebele), 5.

Coccinella 7-punctata bruckii Mulsant

Japan (Koebele, No. 1218), 3.

("1218. At Yokohama, Japan, beginning of March 1895, two specimens hibernating amongst a bunch of liliaceous leaves. One on floor of hotel. May 25, 1895, common upon most any aphid in larva state; some of the mature insects already out. Later on, common upon the hop aphids and various others on low plants. Bred parasite of pupa marked No. 1218, a species of Tetrastichine.")

Coccinella nivicola monticola Mulsant

Boston, Massachusetts, June 29, 1898 (Ormonde), 1; Placer County, California (Koebele), 2. Also 1 female without data belonging to the variety or race *alutacea* Casey.

Coccinella prolongata Crotch

"Washington Territory," 1 female.

Coccinella californica Mannerheim

Sonoma County, California, October (Koebele), 1 female.

Coccinella transversoguttata Faldermann

Oregon (Koebele), 5; Placer County, California (Koebele), 1; Mexico (Koebele, No. 1686), 7; Guadalupe, D. F., Mexico, Nov. 1907 (Koebele), 1; Ecuador (Baron), 1. These are mostly of the form *nugatoria* Mulsant.

("1686. Seven specimens while beating, base of Popocatepetl, 10,000 ft., May 1897, on aphids on *Pinus*.")

Coccinella 9-notata Herbst

Arlington Heights, Massachusetts, July 21, 1896 (Ormonde), 2; Newark, New Jersey, Aug. 16, 1896 (Ormonde), 1; New York (T. B. Ashton), 1; Columbus, Ohio (Koebele), 4.

Coccinella 9-notata degener Casey

Siskiyou, California, July (Koebele), 1.

Coccinella 5-punctata Linnaeus

Waldkirch, Baden, Germany (Koebele), 4.

Coccinella trifasciata Linnaeus

Easton, Washington (Koebele), 2.

Coccinella trifasciata eugenii Mulsant

Siskiyou County, California (Koebele), 1.

Coccinella trifasciata juliana Mulsant

Alameda, California, November (Koebele), 1; Santa Cruz Mountains, California (Koebele), 1.

Coccinella trifasciata subversa Leconte

Oregon (Koebele), 2; Easton, Washington (Koebele), 1; Alaska 1.

Coccinella hieroglyphica kirbyi Crotch

Coccinella tricusps Kirby, 1838 (not Thunberg, 1794).

Minnesota, Sept. 1896 (Wickham), 1 female.

Dobzhansky in his revision of the North American species of *Coccinella* states that the genitalia of *tricusps* are completely like those of *hieroglyphica* Linnaeus. However, I have observed a slight difference in the few specimens that I have compared, but perhaps not enough of a difference to lead one to suppose that *kirbyi* is much more than a subspecies of *hieroglyphica*. The median lobe of the tegmen is a little shorter in *kirbyi* than in *hieroglyphica* and tapers gradually from the broadest part just before the base to a narrower and more acute apex. The ventral surface of the apical part is appreciably tectiform. In *hieroglyphica* this lobe is appreciably less triangular in outline, the sides being more arcuate and the apex blunter. The apex is also just appreciably curled upward and the ventral surface of the apical part is much more depressed than in *kirbyi*. The paramera in *kirbyi* are also slightly more expanded on the apical half.

Coccinella hieroglyphica humboldtiensis Nunenmacher

Siskiyou County, California (Koebele), 2 females. There is also 1 male with the same data in the U. S. National Museum.

The genitalia of *humboldtiensis* are exactly as in *kirbyi*.

Coccinella novae-zelandiae Colenso

New Zealand (Koebele), 6; Paramatta, New South Wales, June 2, 1904 (Koebele), 1 female.

This species of the southern hemisphere corresponds to the holarctic *C. 11-punctata* Linnaeus, and indeed is not very distinct therefrom. I have also seen one male that was collected by O. H. Swezey in Field 39, Oahu Sugar Company, Hawaii, June 1, 1923.

Coccinella transversalis Fabricius

Coccinella transversalis Fabricius, 1781.

Coccinella repanda Thunberg, 1781.*

China (Koebele, No. 1337), 1; Formosa (Koebele, No. 1337), 1; Ceylon (Koebele, No. 1209), 2; Australia (Koebele, No. 17), 31; New South Wales (Koebele, No. 1158), 3; Gordon, New South Wales (Koebele), 1; New Caledonia (Koebele), 3; Fiji (Koebele), 1; and 12 specimens without data, probably from Australia.

("1337. One specimen saved, collected upon pine, Kowloon, Nov. 1895. Amoy, China, Dec. 9, 1895, and Swatow, hibernating. Kowloon, on various plants, not breeding, Dec. 13, 1895. Bred from larva found upon a liliaceous plant infested with aphids and *Dactylopius*, Oct. 11, 1895. At Tamsui, Formosa, beginning of Dec. 1895, one specimen. Many sent to Honolulu during above time."

"1209. One specimen brought to me from Aragam, Dec. 27, 1894. Very common amongst grass in larva, pupa and imago state. Some sent to Honolulu. Out of ten pupae collected but one of these produced the mature insect, and all others chalcid parasites which are mounted and bear this number. Parasite is *Syntomosphyrum* sp."

"1158.") See Koebele note under *Coelophora inaequalis*, var. *9-maculata* (Fab.).

Coccinella leonina Fabricius

New Zealand (Koebele), 1 female.

* *C. transversalis* has a few month's priority as claimed by Mulsant.

COCCINELLINA NEW GENUS

The neotropical species (except *C. transversoguttata* Fald.) which have been referred to *Coccinella* are rather different from the familiar holarctic species, being mostly small, or rather small, more or less oval and less convex species. Structurally they are very similar to *Coccinella* but lack the oblique line of the metacoxal plate, the bounding line curving off very close to the hind margin of the segment. Mesosternum sometimes very slightly sinuate in front medially. Pronotum black with a narrow pale border on anterior and lateral margins and sometimes with two discal spots. The male genitalia of *Coccinellina* are in general similar to those of *Coccinella*, but the median lobe of the tegmen is not modified as in some species of *Coccinella* (i.e., *C. 9-notata* Herbst, *C. transversoguttata* Fald., etc.,) but agrees better with such species as *C. hieroglyphica* Linn. and *C. 11-punctata* Linn. On the dorsal surface of this lobe are two groups or two lines of fine hairs.

The following Central and South American species, all described under *Coccinella*, may be included at least provisionally under *Coccinellina*: *C. ancoralis* (Muls.), *C. arcata* (Muls.), *C. emarginata* (Muls.), *C. eryngii* (Muls.), *C. fulvipennis* (Muls.), *C. lucasii* (Muls.), *C. petiti* (Muls.), *C. pulchella* (Muls.). Of these species *C. emarginata* (*Coccinella emarginata* Mulsant) may be taken as the genotype.

***Coccinellina emarginata* (Mulsant)**

Mexico (Koebele, No. 1688), 2; Morelos, Mexico, October (Koebele, No. 1688), 2; Vera Cruz, Mexico (Koebele), 1.

I have seen examples of this species from many other localities in Mexico, also Nicaragua (Managua and San Marcos), Costa Rica (San Jose, Zarzero and Tilaran), Honduras (Tegucigalpa), Venezuela (Merida) and Ecuador (Mérea and Baños).

("1688. A form that I cannot place, close to *C. sanguinea*. One specimen at Cuautla, Morelos, four on Popocatepetl, Mex., marked with this number.")

***Coccinellina lucasii* (Mulsant)**

Chile (Pfordte), 2.

ADALIA MULSANT

***Adalia bipunctata* (Linnaeus)**

Waldkirch, Baden, Germany (Koebele), 1; Trigny, France, Aug. 1901 (Koebele), 2; New York, 2; Philadelphia, Pennsylvania, Oct. 1903 (Koebele), 2; Columbus, Ohio, Sept. 1903 (Koebele), 1.

***Adalia bipunctata*, var. *quadrimaculata* (Scopoli)**

Waldkirch, Baden, Germany (Koebele), 2; Marin County, California (Koebele), 1; Hayward, Alameda County, California, on *Coccus hesperidum* Linn. (Koebele), 1; Alameda County, California (Koebele, No. 24), 1; Placer County, California (Koebele), 1; Siskiyou County, California (Koebele), 1; Davenport, Tulare County, California, 6400 feet, May, 1903, on willow (Fuchs), 4.

***Adalia frigida* (Schneider)**

From the work of Miss Miriam A. Palmer in Colorado on the heredity of *Adalia* we may safely conclude that most if not all the endemic North American forms of this genus may be referred to the holarctic species *A. frigida* (Schneider). The forms are in no sense geographic races but rather genetic forms or color phases and more or less perfectly segregant in heredity.

The following forms are found in the Koebele collection:

var. *parvula* Weise

Massachusetts, 1; Michigan, 1.

var. *melanopleura* (Leconte)

Placer County, California (Koebele), 1; Easton, Washington (Koebele), 2.

var. *immaculata* Johnson

Oregon (Koebele, No. 20), 1; Alameda, California (Koebele, No. 21 and 501), 1.

var. *annectans* (Crotch)

Oregon (Koebele), 1; Siskiyou County, California, July and August (Koebele, No. 19), 2; Placer County, California (Koebele), 2; Eldorado County, California (Koebele), 1; Alameda, California (Koebele), 1; Argus Mountains, California, on *Pinus monophylla* May 1891 (Koebele, No. 20), 1.

***Adalia 10-punctata* (Linnaeus)**

Waldkirch, Baden, Germany (Koebele), 5. These are mostly of the variety *10-pustulata* (Linnaeus).

APHIDECTA WEISE

***Aphidecta oblitera* (Linnaeus)**

Waldkirch, Baden, Germany (Koebele), 8.

TYTTHASPIS CROTCII

***Tytthaspis 16-punctata* (Linnaeus)**

Berlin, Germany (Weise), 1 male.

SYNHARMONIA GANGLEBAUR

***Synharmonia conglobata* (Linnaeus)**

Yvours, France, July, 1901 (Koebele), 1 female.

CISSELLA WEISE

***Cissella furcifera* (Guérin) (Plate I, Fig. 2)**

One specimen of this Australian species in wretched condition and without data, except the number 2595.

The claws are entirely missing, but one antenna is complete and ten-jointed by partial fusion of the normal last two joints. Joints 3 and 4 about as long as thick. Joints 5 and 6 subequal, somewhat longer than either 3 or 4, and about one and one-third times longer than wide. Joints 7 and 8 subequal as to length, each somewhat longer than either 5 or 6, 7 being hardly wider at apex than the preceding joints, but 8 distinctly so. Joint 9 somewhat longer than 8, widened toward apex, and about one and one-third times longer than wide. Joint 10 (10 and 11 fused) oblong, somewhat more than one and one-half times longer than wide, and slightly contracted at each end, with the apical margin very slightly oblique. On one surface the suture separating joints 10 and 11 may be distinguished, both joints being distinctly broader than long and 10 distinctly larger than 11. The markings are in close accord with Mulsant's description.

Crotch's recognition of a species of *Verania* as *furcifera* was of course not sound.

COCCINULA DOBZHANSKY

Coccinula 14-pustulata (Linnaeus)

Bas-Alpes, France, 1901 (Koebele), 1.

HARMONIA Mulsant

The type of *Harmonia* has never been cited definitely to my knowledge. Four species were originally included, and of these *Coccinella marginepunctata* Schaller (*C. 4-punctata* Pontoppidan) is here selected as the type. On this basis *Ptychanatis* Crotch, 1874 (type, *Coccinella axyridis* Pallas) and *Callineda* Crotch, 1871 (type, *Coccinella 16-notata* Fab., per Rye, 1873)* fall as synonyms. Also most of the species included in *Leis* Mulsant, 1850 (type, *Coccinella dimidiata* Fab., per Crotch, 1874) and in *Stictoleis* Crotch, 1874 (orthotype, *Coccinella coryphaca* Guérin) must be assigned to *Harmonia*, although I have had no opportunity to investigate the genotypes. The genus is very widely distributed in Europe, Asia, Africa and Australia but does not occur in the New World.

Harmonia axyridis (Pallas)

China (Koebele, No. 1312), 14; Hongkong, China (Koebele, No. 1312), 6; Formosa (Koebele, No. 1312), 1; Japan (Koebele, No. 1235), 56. Also 66 specimens without data, but presumably from China.

This large series shows the usual well known variation in markings.

("1235. Very numerous at Miyanoshita, Japan, April 6-8, 1895, upon various trees and sometimes in lots, yet hibernating. Also Atami a few days later, here already laying eggs with aphids on rose. A variable insect, many pinned. Quite common upon aphids on maple at Yokohama, April 20, 1895. Many sent to Honolulu.

"Became very numerous the end of May and beginning of June, when the second brood began to appear. Feeding upon any kind of aphid and *Lachnus*, a bluish aphid upon *Podocarpus*, *Kermaphis pini* on pine. Sept. 12, 1895, at Tumato, Japan, upon willow infested with aphids, large numbers of pupae were observed, and at Nikko on Sept. 19, 1895, upon walnut infested with a pale spiny aphid and a leaf-hopper (*Ledra*?), larvae of all sizes were still present and beetles [that were] collected oviposited many eggs."

"1312. Very abundant upon *Pinus sinensis*, at Hongkong, China, Oct. 21, 1895, and here feeding upon *Lachnus* infesting these trees. Eggs were numerous but since 1306 [*Sospita chinensis* Muls.] and 1307 [*Bothracalva albolineata* Schônkh.] were also numerous it cannot be stated to which they belonged. Bred from larvae on *Lachnus*. Also at Tamsui, Formosa, upon pine infested with *Lachnus*, Dec. 6, 1895.")

Harmonia conformis (Boisduval)

Australia (Koebele, No. 3), 15; New South Wales (Koebele, No. 1003), 1; Bundaberg, Queensland (Koebele), 2.

("1003. Common all over S. Australia, Victoria, New South Wales and southern Queensland, chiefly feeding upon Aphidae, yet later in the season doing good work on the woolly aphids. Bred same parasite from this beetle as found in Europe

* Crotch in his Revision (1874) selected *Callineda decussata* Crotch as the type of *Callineda*. As this species is not congeneric with the type selected by Rye (Zool. Rec. 8, p. 329), I propose *Rhopalonedra*, n.n., for *Callineda* Crotch, 1874 (not Crotch, 1871).

and America. At Mt. Victoria, N. S. W., March 28, 1892, breeding upon *Eriococcus leptospermi* Mask.

"Observed eggs on orange infested with aphid at Honolulu, March, 1894, from specimens liberated on tree two weeks previous.")

Harmonia antipodum (White)

New Zealand (Koebele, No. 486), 4.

("486. Found these insects quite abundant, feeding on *Ctenochiton viridis* Maskell, near Auckland, N. Z. They were at the time, Feb. 23, 1889, in all stages from eggs to the mature insects.")

Harmonia testudinaria (Mulsant)

Australia (Koebele, No. 19), 5, two of these with the additional data: Toowoomba; Brisbane, Queensland (Koebele), 10; Bundaberg, Queensland (Koebele), 2.

("1019. At West Maitland, New South Wales, Nov. 5, 1891, upon peach trees infested with aphid, a few specimens only, one pair in copula. Also at Brisbane upon orange and *Ficus* sp., Nov. 12, 1891. At Toowoomba, Nov. 15, 1891, numerous upon nettle tree and myrtle. Upon these one very fresh and soft specimen found, yet no larvae observed. One larva upon aphid on orange at Parramatta.")

Harmonia bicolor (Blackburn)

Cairns, Queensland (Koebele), 2; Geraldton, Johnston River, Queensland (Koebele, No. 1183), 2.

("1183. With the foregoing [1182, *Microcaria jansoni* Crotch] at the same place and food [at Geraldton, Q., Aug. 10-15, 1894, upon *Hibiscus* tree, badly infested with a Psyllid], one specimen apparently different, marked 1183. One specimen at Kuranda, Aug. 21, 1894, upon young tree with *C. repanda*.")

Harmonia 16-notata (Fabricius)

China (Koebele, No. 1342), 2 females.

("1342. Two specimens at Kowloon, Nov. 18, 1895, upon *Psidium* infested with *Pulvinaria psidii*.")

Harmonia arcuata (Fabricius)

China (Koebele, No. 1341), 8; Fiji (Koebele), 8; Australia (Koebele, No. 53 and 1053), 5; Toowoomba, Queensland (Koebele, No. 53), 2; Cairns, Queensland (Koebele), 5.

("1053. At Broadwater, Richmond River, New South Wales, Jan. 10, 1892, upon a fern growing in swampy ground and infested with *Lecanium*, larvae, pupae and imagoes, in vial No. 53. April 6-15, 1892, at Toowoomba, Queensland, a number of specimens feeding upon aphid on young orange trees, variable. At Levuka, Fiji, breeding upon *Aleyrodes* upon taro leaves (*Colocasia* sp.), Jan. 19, 1892. At Cairns, Queensland, beginning of August 1894, on various plants, common near sea-shore upon a morning-glory, feeding upon Cicadinae.")

"1341. First specimen found upon a weed, second upon orange infested with *Lecanium*, *Pulvinaria*. More here and breeding upon *Hemerocallis* infested with *Pulvinaria*, *Dactylopius* and *Lecanium*, Kowloon, November 1895. Many specimens, Swatow, China, Dec. 11, 1895.")

Harmonia 4-punctata (Pontoppidan)

One female of this European species without data, belonging to the var. *16-punctata* (Fabricius).

MULSANTINA WEISE

Mulsantina was proposed by Weise (1906) as a new name for *Cleis* Mulsant, 1850, p. 162 and 208 (type *Cleis mirifica* Mulsant), not *Cleis* Mulsant, 1850, p. 130 and 135 (= *Clynis* Mulsant), nor *Cleis* Guérin, 1832. *Pseudocleis* Casey, 1908 (orthotype, *Coccinella picta* Randall) is a synonym.

Mulsantina picta (Randall)

Wisconsin (Wickham), 2; Placer County, California (Koebele), 2.

Mulsantina picta minor (Casey) (Plate I, Figs. 3, 4, 5)

Alameda, California (Koebele, No. 27 and 50), 6, one of these from a larva feeding on pine aphids at Hayward, issuing Dec. 1, 1893; Oregon (Koebele, No. 25), 2; Easton, Washington (Koebele, No. 26), 4; Mexico (Koebele, No. 501 and 1647), 18; Morelos, Mexico (Koebele), 1; Guadalupe, D. F., Mexico, Nov. 1907 (Koebele), 1.

("501. Dec. 25, 1889, at Alameda upon *Pinus insignis* blown over by wind and thickly infested with *Lecanium* sp., which were yet small, was found very abundant, a variable coccinellid. Also collected at Sisson upon pine, but at this locality no scales were observed, but large aphids were abundant. No larvae or pupae were found at this date, except old pupae, which were probably those of *Chilocorus bixulnervus* Muls., which insect was also present in large numbers.

"July 13, 1893, found upon orange infested with *L. olcae* and *L. hesperidum*, at Hayward, Alameda Co., Calif., not common.

"Very numerous in Oregon, during April-June, 1893, upon fir trees infested with a white coccid. Bred on *Crataegus* infested with *Lecanium* sp. and aphids, feeding on the latter.

"Bred from larvae feeding upon *L. olcae* at Hayward, Sept. 16, 1893. This specimen appears to differ—punctuation of elytra finer.

"Common at base of Popocatepetl, Mex., in many varieties, upon pine and fir trees infested with aphids."

(For notes on one specimen confused under No. 1647, see following species.)

Mulsantina mirifica (Mulsant) (Plate I, Figs. 6, 7)

Mexico (Koebele, No. 1647), 6. Also two specimens from the same source in the U. S. National Museum.

("1647. Eight specimens of peculiar species, beating, at Oaxaca, Mexico, August 18-22, 1897.")

Mulsantina mirifica, var. **lynx** (Mulsant) (Plate I, Fig. 8)

Mexico (Koebele, No. 1649), 3. Also two more with same data in U. S. National Museum.

("1649. Four specimens collected, beating, at Oaxaca, Mex., August 17-22, 1897.")

This variety is a little smaller than *M. picta minor* (Casey) more broadly oval and with appreciably finer and sparser punctures on the elytra. The male genitalia of *lynx* are identical with those of *mirifica* and I agree with Crotch in making it only a variety.

Gorham's *Coccinella albopicta* is evidently the same as *mirifica*, as I can not detect anything in his description whereby to separate it. On page 168 of the "Biologia" Gorham remarks that a specimen determined by Crotch as *Cleis lynx* belongs to his *albopicta*.

NEOHARMONIA CROTCH

For the type of *Neoharmonia* Crotch, 1871, Rye (Zool. Rec. 8, p. 329, 1873), selected *Harmonia viridipennis* Mulsant among the twelve described species originally included. *Agrabia* Casey, 1899 (haplotype, *Harmonia cyanoptera* Mulsant) and *Neoharmonia* Casey, 1899 for which I hereby select *Coccinella venusta* Melsheimer, as type, are synonyms.

Neoharmonia cyanoptera (Mulsant)

Douglas County, Arizona, Aug. 1904 (F. H. Snow), 1 female.

Neoharmonia venusta (Melsheimer)

Virginia (Beyer), 1 male.

SOSPITA MULSANT

Sospita chinensis Mulsant

Hongkong, China (Koebele, No. 1306), 3; and 115 specimens without data but presumably from Hongkong.

("1306. At Hongkong, China Oct. 29, 1895, upon *Pinus sinensis*, quite common a coccinellid, apparently found previously in Japan. Feeding upon *Lachnus*.")

MYRRHA MULSANT

Myrrha 18-guttata (Linnaeus)

Waldkirch, Baden, Germany (Koebele), 2 females.

CALVIA MULSANT

Calvia 10-guttata (Linnaeus)

Japan (Koebele, No. 1237), 3 females.

("1237. Few at Miyanoshita and more at Atami, April 1895, upon various trees and not upon any particular food. Some sent to Honolulu. At Yokohama in larva state upon alder infested with mildew.")

ANISOCALVIA CROTCH

Anisocalvia 14-guttata (Linnaeus)

Waldkirch, Baden, Germany (Koebele); 2 females; Japan (Koebele, No. 1237), 1 female. (See Koebele note under preceding species.)

The following varieties of *14-guttata* were recognized in the Koebele collection:

var. *scutulata* (Weise)

Oregon (Koebele, No. 15), 1 female.

var. *12-maculata* (Gebler)

Oregon (Koebele), 2 males.

var. *vancouveri* Casey

Oregon (Koebele, No. 16), 7.

ANATIS MULSANT

Anatis ocellata (Linnaeus)

Waldkirch, Baden, Germany (Koebele), 7; Oregon (Koebele), 3.

The Oregon specimens are quite typical both in form and markings. The sutural pubescence at apex of elytra is well developed.

Anatis ocellata halonis Lewis

Japan (Koebele, No. 1262), 1 male.

("1262. One specimen upon oak infested with *Lachnus* at Gifu, Japan, May 2, 1895.")

Anatis ocellata mali (Say)

One male without data, but presumably from the eastern part of the United States.

Mali is quite distinct from *15-punctata*, but I believe it is only a race of the holarctic *ocellata*. It has the sutural pubescence of *ocellata* and the male genitalia are identical.

Anatis 15-punctata (Olivier)

Boston, Massachusetts, July 16, 1896 (Ormonde), 1 male; Providence, Rhode Island, May 21, 1898 (Ormonde), 1 male.

Anatis rathvoni (Leconte)

Alameda, California (Koebele), 1; Siskiyou County, California (Koebele), 3; Placer County, California (Koebele), 2; Easton, Washington (Koebele), 1; Oregon (Koebele), 1.

NEOMYSIA CASEY

Contrary to Casey's opinion I find that North American species of *Mysia* Mulsant are quite congeneric with the European genotype, and in fact, so closely related that it would be better to consider them races of *oblongoguttata*. As *Mysia* is a preoccupied name, *Neomysia* Casey, 1899, will hold, and *Paramysia* Reitter, 1911, falls as a synonym.

Neomysia oblongoguttata (Linnaeus) (Plate I, Fig. 9)

Waldkirch, Baden, Germany (Koebele), 3 females; Oregon (Koebele), 1 male.

The Oregon specimen is certainly not separable from European material of *oblongoguttata*. I have compared the genitalia with those of several European males and find them practically identical. *N. hornii* (Crotch) was described from Oregon and the above specimen agrees so closely with the description that the conclusion is inescapable that *hornii* must fall as a synonym of *oblongoguttata*. *N. hornii* was described as being entirely testaceous red except that the sides of thorax are broadly whitish and the mesepimera white. The Koebele specimen has the elytra streaked with a paler color, just as is commonly seen in many European specimens, but I do not believe that this deviation from the condition found in type of *hornii* is of much importance.

Neomysia oblongoguttata caseyi new subspecies

Specimens from California commonly referred to *hornii* differ somewhat in coloration and slightly in the male genitalia. The pronotum lacks a well defined median dark area, but sometimes there is a nubilous and more or less broken darker M-shaped mark. Typically the pron-

tum and elytra are almost uniformly reddish. The elytra, however, may have three reddish vittae on a paler ground, of which the two inner ones are broader and unite near apex.

In the type series from Eldorado County, California, three have a more or less definite M-shaped mark on thorax and vittate elytra. Two are similar but have the two inner vittae more or less expanded and confluent so as to cover almost all of the area of the suture. The other five have the elytra nearly uniformly colored, although sometimes a little paler on outer margin. One of these, which is much paler in color than the others, has a feeble M-shaped mark on the thorax, while the other four (including holotype) have hardly any trace of it.

The median lobe of the tegmen (male aedeagus) as seen from above is narrow but distinctly less acicular than in *oblongoguttata*. It expands slightly from the middle to apical third, whence it tapers gradually to an acute point. In *oblongoguttata* it is uniformly narrow. As seen from the side this lobe is considerably less deep in *caseyi* than is *oblongoguttata*.

Described from ten specimens (holotype and paratypes) in the Koebele collection from Eldorado County, California.

***Neomysia oblongoguttata interrupta* Casey**

Argus Mountains, Inyo County, California, on *Pinus monophylla*, May 1891 (Koebele, No. 17), 3.

The median lobe of the tegmen in this race is similar to that of *caseyi*, but is slightly narrower and attains its greatest width at distance of about one-fourth the length of lobe from apex instead of about one-third.

This race is widely distributed, occurring in Southern California, Arizona, New Mexico, Colorado, and Utah. In some localities in Arizona, New Mexico, and Colorado the inner vitta is more or less completely confluent at apex with the median vitta. This character used by Casey to separate *hornii* (not Crotch) from *interrupta* is therefore too fluctuating to be of much value. It may even be present in the race *caseyi* above described, but that shows a distinct tendency toward an immaculate condition of the elytra.

A further peculiarity of this reddish-vittate race not mentioned by Casey is the presence of an additional spot or streak intercalated between the bases of the median and subsutural vittae. This spot in the vittate specimens of *caseyi* is not very distinct and is confluent with the subsutural vitta.

Specimens from Hood River, Oregon (Hubbard and Schwarz), and Bear Paw Mountains, Montana (Hubbard and Schwarz), are not typical but perhaps are better referred to *interrupta* than elsewhere. They have vittate elytra, with the intercalated mark. Four out of twelve specimens from Bear Paw Mountains have black instead of reddish markings and resemble *randalli* Casey, but the male genitalia are similar to those of *interrupta*. In the true *randalli* from the Lake Superior region the genitalia have the median lobe of tegmen of uniform width as in *pullata*, but more slender, yet not so acicular as in *oblongoguttata*.

***Neomysia oblongoguttata subvittata* (Mulsant)**

Placer County, California, June (Koebele), 5; Oregon (Koebele), 1; Easton, Washington (Koebele), 1.

This race differs from all the others in the broad form and ventricose elytra. It has been commonly identified as *subvittata* (Mulsant) although redescribed by Casey as *oregona*.

The elytra have more or less developed black or fuscous vittate markings. The dark median area of the pronotum is much narrower than in *pullata* and varies from red-brown to black. In the former case it may be uniformly colored or margined

laterally with black either partially or completely. Sometimes the dark area encloses two basal pale spots, thus vaguely indicating an M-shaped mark seen in other races. In one specimen the pronotum has two basal black spots, the remainder of the median dark area feebly indicated by a slightly darker color than the sides. The pale side areas are always much larger than in *pullata* and show no trace of an enclosed dark spot. The dark vittae of the elytra vary greatly, but are usually more or less incomplete or broken. The median vitta is sometimes considerably broadened and confluent at apex with the outer vitta. The subsutural vitta is always short and free.

In form and markings this is removed about as far as possible from typical *oblongoguttata* but it approaches it most closely of all the North American races in the form of the male aedeagus. I have examined the genitalia of three males and they all differ slightly. In one male from Placer County, California, the median lobe of the tegmen is virtually as in *oblongoguttata*, being if anything a little more elongate and acicular. In another male from the same locality, the median lobe is slightly widened between the middle and apical fourth where it begins to taper to the apex. In a third specimen, taken from sea-drift, Manzanita, Oregon (S. E. Keen) the median lobe is uniformly still wider from base up to a point near the apex where it begins to taper (virtually as in *pullata*). I am unable to say at present which of these is most representative of the race as a whole.

***Neomysia oblongoguttata pullata* (Say)**

New York, 1 female.

***Neomysia gerstäckeri* (Mulsant)**

Uruapan, Michoacan, Mexico, July, 1906 (Koebele), 1 female.

CYCLONEDA CROTCII

The genus *Cycloneda* should be restricted to *sanguinea* and allies with immaculate elytra. The numerous neotropical species commonly referred here are much more closely allied to *Neda* than to *Cycloneda*.

Over twenty years ago I had opportunity to assemble living material of all three of the North American species of *Cycloneda* (*sanguinea*, *munda* and *polita*) and made numerous reciprocal crosses. Only newly emerged or virgin females were used in the experiments and whenever they were mated with males of the other two species they always remained sterile. There are only slight differences in the male genitalia of the three species, but these differences are apparently constant, and in view of the sterility between the species I believe that they are perfectly distinct.

***Cycloneda sanguinea* (Linnaeus)**

Vera Cruz, Mexico (Koebele, No. 1641), 2; Morelos, Mexico (Koebele, No. 1641), 8; Orange, California (Koebele), 1.

("1641. Collected at Orizaba and Oaxaca, Mexico, where one specimen was bred from pupa found under pecan nut infested with *Lachnus*. Cuautla, Morelos, Mexico.")

***Cycloneda munda* (Say)**

New York City, New York (Ormonde), 1; and 2 without data.

Cycloneda polita Casey

Oregon (Koebele, No. 23), 2; Easton, Washington (Koebele), 2; Siskiyou County, California (Koebele), 1.

Cycloneda polita flava new subspecies

Like typical *polita* but perhaps averaging a little smaller and more or less testaceous yellow instead of brilliant scarlet. It is thus much like the eastern *munda* but much smaller. The markings of the pronotum as in *polita* and *munda*, the differences cited by Casey between the two being rather inconstant.

Length, 3 to 4.25 mm.

Described from 6 specimens (holotype and paratypes) from Alameda, California (Koebele), and 3 specimens (paratypes) from Santa Cruz Mountains, California (Koebele, No. 22). All except one paratype from Alameda are in the Koebele collection. The one exception in collection of Citrus Experiment Station.

OLLA CASEY**Olla V-nigrum** (Mulsant)

Coccinella abdominalis Say, 1824 (not Thunberg, 1794).

Harmonia V-nigrum Mulsant, 1866.

Cycloneda sayi Crotch, 1871, n.n. for *abdominalis* (Say).

Other early names, such as *binotata* (Say) and *oculata* (Fab.), that have been used sometimes for this species, or its dimorphic form, are apparently not applicable.

Santa Cruz Mountains, California (Koebele), 3; Eldorado, California (Koebele), 2; Arizona (Koebele, No. 2430), 1; Mexico (Koebele, No. 1652), 1; Morelos, Mexico (Koebele, No. 1652), 2; Sonora, Mexico (Koebele, No. 1652), 10; Honolulu, Oahu (Koebele), 1.

("1652. Three specimens, Cuautla, Morelos, Mexico, August 1897, all mounted. Base of Popocatepetl, Mex., May 1897, on pine infested with aphids, 10,000 feet. California and Hawaiian Islands, where it has been common for some twenty years.")

Olla V-nigrum, var. **plagiata** Casey

Santa Cruz Mountains, California (Koebele), 1; Los Angeles County, California, July (Koebele), 2; Tucson, Arizona (Kunze & Wickham), 2; Sonora, Mexico (Koebele, No. 1681), 1.

("1681. Two specimens feeding on *Alecyrodes*, Hermosillo, Sonora, Mex., April 1897.")

PARANEDA NEW GENUS

This genus is proposed for some of the neotropical species hitherto referred to *Cycloneda*, especially *pallidula* (Mulsant). The type is *P. viridescens* n. sp. described below.

Frons less than twice as wide as diameter of eyes, with the inner orbits convergent above. (Wider in *Olla* and *Cycloneda*, with inner orbits parallel.) Eyes rather coarsely faceted. Antennae strongly clavate, the club large, not elongate, with the last joint slightly longer than wide and obliquely truncate. Prosternal process bicarinate. Mesosternum moderately emarginate medially in front. Epipleura rather less than twice as wide as space between middle coxae, which are rather broadly separated. Epipleura slightly subfoveate to receive apex of hind femora. Metacoxal line curving outward just in front of the hind margin of the segment, the oblique line absent. Elytral punctures very fine. Form orbicular, very convex. Elytra unicolorous. Pronotum pale at the sides and darker medially, the two shades separated by a

more or less distinct black line which curves outward to the basal angles and then sometimes extends forward along the side margins for a short distance.

*Cycloneda rubida** and *Paraneda pallidula* have been confused more or less and were considered by Gorham to be varieties of one species, but they are quite distinct, although it is rather difficult to discriminate them in every case. *Rubida* differs from *pallidula* in having the frons very narrow, no wider than diameter of the eyes, the latter a little more coarsely faceted; claws small, much shorter than in *pallidula*, or hardly longer than the basal quadrate tooth; mesosternum rather less emarginate in front medially; and the male genitalia decidedly different. *Rubida* is also more orbicular than *pallidula*. The color is deep red, varying at least after death to flavous. The darker color at the middle of the pronotum is not so broad as in *pallidula*, the line of demarkation more curvilinear, curving outward in front slightly as well as behind, but hardly reaching to the posterior angles and never continued forward as a dark line on the exterior margins. Of *rubida* I have seen the following material in the U. S. National Museum: 2 from Bugaba, Panama (Champion); 2 from Cayuga, Guatemala (Wm. Schaus); and 2 from Cacao Aguas, Alta Vera Paz, Guatemala (Schwarz and Barber).

Pallidula was described from Cayenne and Brazil, and *gutticollis* (Mulsant) from South America without a more definite locality. *Gutticollis* has the darker median area of the pronotum bordered with black. *Pallidula* as defined by Mulsant differs in lacking the black border, but it is likely that this is not a constant character and at any rate Crotch and other authors have recognized only one species. Crotch says that *pallidula* "varies a good deal in appearance, being in life a bright green, which pales to a dirty yellow." The Mulsantian descriptions, however, do not give much suggestion of a green color in life. It seems likely that Crotch as well as Gorham have confused two or more species under *pallidula*. Unfortunately I have not been able to see any South American material, but I am able to distinguish two species in material from Central America and Mexico. One of these is described below as *viridescens*. The other is more likely the true *pallidula*. It is represented by one male collected at Cacao Trece Aguas, Alta Vera Paz, Guatemala (Schwarz and Barber). It has the middle of the pronotum and the elytra flavous (without any hint that a green color may have existed in life) and the elytra are not blackened along the sutural margin. The genitalia of the Guatemala specimen differs very decidedly from that of *viridescens*. The aedeagus as a whole is much more elongate. Siphon not twisted at apex, the filament beyond the dorsal flaps much prolonged, nearly as long as the part on basal side of flaps, very slender and becoming exceedingly attenuate at apex. Paramera slender. Median lobe of tegmen about four times as long as wide, with subparallel sides, depressed, but with a slight tectiform swelling on dorsal surface at basal third, the apex deeply emarginate, with tooth on each side of the emargination acutely triangular and twice as long as wide at base.

***Paraneda viridescens* n. sp. (Plate I, Fig. 10)**

Elytra light green, fading after death more or less to a pale testaceous color, or to various pale shades of yellowish, brownish or flavous. Sutural margin of elytra very narrowly blackened. Middle of pronotum dull brownish or reddish, the sides with a broadly oval pale yellowish or creamy area. Border of the darker area marked more or less distinctly with a

* In the Appendix this species is referred to a new genus (*Erythroneda*).

black line, which is curvilinear behind and meets the basal angle where it turns forward and follows the outer margin for a short distance. Anteriorly the black line becomes straighter and fails to attain the anterior margin by a brief interval. The head, under surface of body and the legs, more or less reddish brown, but the epipleura of prothorax and of elytra much paler and the mes- and metepimera are whitish. Length about 5.9 to 7 mm., width about 4.9 to 5.5 mm.

Aedeagus in *viridescens* much less elongate than in *pallidula*. Siphon not slender, not produced into a filament beyond the rather elongate membranous flap and strongly twisted near apex. Paramera distinctly widened toward the apex. Median lobe of tegmen about three times as long as wide, with parallel sides and a high tectiform ridge on the basal two-fifths of dorsal surface. Basal part of this lobe very thick dorso-ventrally which is not the case in *pallidula*. Apex of lobe with a broad rounded emargination, the tooth on each side of emargination obtuse and hardly longer than wide at its base.

Described from the following material: 6 specimens (holotype and paratypes), Eldorado, Sinaloa (S. E. Flanders), two paratypes in U. S. National Museum, the others including holotype, in collection of Citrus Experiment Station; Mazatlan, Sinaloa (Koebele), 1 paratype in U. S. National Museum; Colima, Colima (Conradt), 1 paratype in U. S. National Museum; Oaxaca (Koebele), 2 paratypes, one in Koebele collection, one in U. S. National Museum. These localities are all in Mexico.

The following material in the U. S. National Museum is probably referable to *viridescens* but the series I believe includes no males, so that the allocation is somewhat doubtful: 5 from Santiago de Maria, Salvador, on coffee (K. A. Salman); 2 from Tucurrique, Costa Rica (Schild & Burgdorf); and 2 from San Jose, Costa Rica (J. Fid. Tristan).

NEDA MULSANT

Neda marginalis Mulsant

Mexico (Koebele, No. 1570), 3; Morelos, Mexico (Koebele, No. 1570), 8.

("1570. Found at Cuautla, State of Morelos, and preying upon a chrysomelid larva infesting a tropical deciduous tree, *Datura*. Specimens mounted and others sent to Washington to be tried upon North American chrysomelid larvae. Larvae of many sizes were found, yet no eggs. Collected July 1-3, 1897. A month previous those trees had no leaves.

"At Oaxaca, Aug. 17-22, pupae found quite numerous under Brazil and walnut trees (pecan-Castilian) infested with *Lachnus* sp., also larva observed amongst lice. A small dipterous larva issuing, numerous, from pupae. Issued end of September, a *Phora*."")

EGLEIS MULSANT

The Australian species here associated are plainly congeneric, but should probably be segregated from *Egleis* proper, which is South American. I have not seen any of the American species of the genus and consequently I am not able to point out any distinguishing characters for the Australian group.

Egleis kingi (Macleay) (Plate I, Fig. 11)

Australia (Koebele), 2.

Egleis delta Weise (Plate I, Fig. 12)

Australia (Koebele, No. 57), 1; Brisbane, Queensland (Koebele), 1; Cairns, Queensland (Koebele), 1.

("1057. One specimen collected on road from Lismon to Brunswick, New South Wales, upon orange tree, on aphid.")

Egleis edwardsii Mulsant (Plate I, Fig. 13)

Australia (Koebele, No. 31), 2; Brisbane, Queensland (Koebele), 1.

Pascoei Crotch is a synonym of *edwardsii*.

("1031. Three specimens, one of them newly hatched, upon orange at Parramatta, New South Wales, Nov. 23, 1891, no doubt feeding upon aphid. One specimen at same place, Dec. 25, 1891. Another at same place Sept. 27, 1894.")

Egleis barronensis (Blackburn) (Plate I, Fig. 14)

Australia (Koebele, No. 1161), 28; Cairns, Queensland (Koebele, No. 1161), 11.

("1161. Quite numerous upon orange and lemon trees at Kuranda, Queensland, and feeding on orange aphid. Also at Kamerunga and Geraldton, Johnston River.")

ARCHAIONEDA CROTCH

Archaioneda tricolor fijiensis Crotch

Fiji (Koebele), 12.

I have not been able to check the identity of this with typical *tricolor* (Fabricius).

CLEOBORA MULSANT

Cleobora mellyi Mulsant

Victoria (Koebele), 1; also one without data.

VERANIA MULSANT

Verania flavovittata Crotch (Plate I, Fig. 15)

Bundaberg, Queensland (Koebele and Perkins), 8.

In this species there are two broad black vittae on the disk of each elytron, uniting at the callus, the inner one oblique, joining or nearly touching the sutural vitta appreciably behind the middle. The other, usually but not always, wider than the inner one, extending straight back parallel with the margin and ending at the apical sixth, where it is rarely joined to the sutural vitta by an expansion of the latter.

I have seen it labeled incorrectly as "*furcifera* Guérin" in some collections.

Verania frenata (Erichson)

Australia (Koebele, No. 4 and 490), 6; Bundaberg, Queensland (Koebele, and Koebele & Perkins), 12; also 10 without data.

("490. Coccinellid on aphid resembling *Kermes* on pine, *Kermaphis pini* Koch.

"Found this insect abundant at Toowoomba, Queensland, Jan. 1, 1889, upon Acacia infested by a coccid resembling that infesting pine trees in Australia and New Zealand. No larvae were observed.")

Verania lineola (Fabricius)

Australia (Koebele, No. 54), 1; Sydney, New South Wales (Koebele, No. 54), 1; Brisbane, Queensland (Koebele), 1; Bundaberg, Queensland (Koebele), 1; Cairns, Queensland (Koebele), 6; Fiji (Koebele), 5.

("1054. Common everywhere from Clarence to Tweed River and very abundant at the latter place, in all stages, breeding upon aphid on maize. One of the larvae

had killed a nearly grown larva of *Heliothis armigera* while coming out of a corn cob.

"New Caledonia; Fiji. Parramatta, one. Brisbane. Sent to Honolulu.

"Everywhere around Cairns, Queensland, July 1904. Bred *Centistes americana* and parasite on larva as well. Found quite numerous at Hambleton and Mulgrave, Cairns, Queensland, July to September, 1904, on sugar cane. Badly parasitized in larva state by chalcid. Specimens sent to Honolulu. Also Bundaberg, Queensland, September to November 1904, also badly parasitized. Found on various plants and trees, not numerous. Always a few obtained for Honolulu.")

Verania discolor (Fabricius)

China (Koebele, No. 1330), 2; also 36 specimens without more definite data than the number; Hongkong, China (Koebele, No. 1330), 4; Anuradhapura, Ceylon (Horn?), 1.

("1330. Two specimens at Kowloon, Hongkong, Nov. 3, 1895, one on *Psidium* infested with aphids, second on *Celtis*, also on aphids. Numerous on *Psidium*, etc. infested with *Pulvinaria psidii*).

"Swatow, China, Dec. 11, 1895, hibernating in large numbers in screw palms, etc., a few specimens upon *Brassica nigra* (mustard) infested with aphids.")

MICRASPIIS CHEVROLAT

Micraspis striata (Fabricius)

Camerun, 1.

Micraspis cardoni (Weise)

Ceylon (Koebele, No. 1213), 2.

Weise placed this in *Verania*, but the orbicular form and broad epipleura agree much better with *Micraspis* (*Alesia* authors).

("1213. One specimen at Kandy, Jan. 4, 1895, upon jack fruit tree (?), where larva was observed. Nenra Eliya.")

PROPYLEA MULSANT

Propylea 14-punctata (Linnaeus)

Yvours, France, Aug. 1900 (Koebele), 1; Waldkirch, Baden, Germany (Koebele), 8.

Propylea 14-punctata japonica (Thunberg)

Japan (Koebele, No. 1236), 13; China (Koebele, No. 1339), 2.

The two from China and two from Japan belong to the var. *dionea* (Mulsant); five from Japan to the var. *tessellata* (Weise) and the remainder to the var. *ancora* (Weise).

("1236. Miyanoshita and Atami upon various plants, not yet found upon any particular food aphid. Sent to Honolulu.")

"1339. One specimen only upon *Paliurus ramosissimus* Poir, Kowloon, November 1895. One specimen on screw palm, Swatow, Nov. 12, 1895.")

PROTOCARIA NEW GENUS

Allied to *Propylea* Mulsant and similar in size, form and appearance. Epipleura of elytra distinctly more descending externally. Legs shorter, the hind pair reaching to middle of

epipleura (almost to outer edge in *Propylea*). Femora broader, the claws smaller, with a smaller rounded basal tooth. Anterior edge of mesosternum weakly emarginate in middle (rather deeply emarginate in *Propylea*). Metasternal lobe between middle coxae narrowed to a more or less obtuse point (broadly truncate in *Propylea*). Coxal plate of first ventrite with a distinct oblique line (the line faint or absent in *Propylea*). Antennae considerably shorter, joints 3 to 6 being at most hardly more than twice as long as thick, 7 and 8 hardly longer than wide, the eighth strongly widened toward apex and rather distinctly forming a part of the club, joints 9 and 10 broader than long, and the last joint about as long as wide and obliquely truncate. (In *Propylea* antennal joints 3 to 6 almost three times as long as thick, 7 and 8 about twice as long as thick, 9 and 10 distinctly longer than wide, and the last joint about twice as long as wide and rounded at apex.) Median process of prosternum with two parallel carinae, lying close together and reaching about three-fourths of the distance to anterior margin of segment. Prothoracic foveae practically as in *Propylea*.

Median lobe of tegmen depressed, nearly three times as long as wide, the lateral margins parallel at base, converging, however, in apical third, but the apex broadly truncate and strongly curved upward. Width of the apical truncation of this lobe about one-third of the greatest width of lobe. Dorsal surface of lobe well rounded from side to side on the basal half. Paramera rather short and stout, almost straight, well separated from each other at base, and slightly thickened at apex. They reach nearly to the apex of the median lobe and are ciliate on inner margin nearly to the base and on outer margin nearly to the middle. Siphon short, moderately slender, abruptly narrowed in the apical eighth and having the extreme apex abruptly bent downward in a right angle, with a minute membranous expansion.

Genotype: *Protocaria scalaris* n. sp.

***Protocaria scalaris* n. sp. (Plate I, Fig. 16)**

Testaceous yellow, the vertex, large mark on pronotum, elytra except pale marking, and under parts black, but epipleura of elytra and of prothorax, the mesepisternum and legs, excepting middle and hind coxae, pale. The prothoracic mark variable. In one female paratype the pronotum is largely black, with the lateral margins, broadly in front, narrowly in rear, pale. The black area in this specimen reaches the anterior margin and is slightly notched in the middle anteriorly. In the other types the black area is less extensive and does not quite attain the anterior margin and is more or less bilobed meso-anteriorly. These anterior lobes either almost squarely truncate or obliquely rounded, depending on whether the median pale notch is deep and narrow, or short and spreading. Outer margin of the black lobes meeting the basal black area more or less squarely. The pale side margins very narrow behind, and more or less abruptly and greatly widened in front. Elytra with lateral margin broadly and three more or less oval or round spots on the disk, yellow, producing a scalariform design. Anterior spot touching basal margin, smaller and more triangular than the other two, rather more closely approaching the suture than the humerus. Second spot largest, before the middle (its hind margins at or slightly before the middle), and usually more or less oblique. Posterior spot placed half way between middle and apex and in Japanese paratypes more rounded than the others. These spots vary considerably in size. In the type and allotype (Formosa) they are large, or wider than the black intervals between them, and both the middle and posterior spots are oval and oblique. The yellow lateral margin undulate within, widest at the base and at the middle. The widening at the middle forms a deep broadly rounded indentation in the black area opposite the interval between middle and posterior spots. In one paratype (Japan) the black area extends into the yellow lateral margin in two definite broad lobes, almost directly opposite the middle and posterior discal spots, and almost dividing the yellow border into three spots. Frons and pronotum very finely and closely punctured, elytra much more distinctly and more sparsely punctured. Length, about 3 to 4 mm.

The Japanese specimens have elytral puncturation usually slightly stronger, the three discal spots smaller, with the posterior one more rounded and less oblique than in the Formosan type.

Formosa (Koebele, No. 1238), 1 ♂, 1 ♀ (holotype ♂ and allotype); Japan (Koebele, No. 1238), 2 ♀ (paratypes); and Gifu, Japan (Y. Nawa), 1 ♂, 1 ♀

(paratypes). Allotype and one paratype (Japan) in Koebele collection, the remainder in the U. S. National Museum.

("1238. Two specimens only, Atami, Japan, April 1895. Three specimens at Tamsui, Formosa, December 1895.")

CARIA MULSANT

Caria dilatata (Fabricius)

China (Koebele, No. 1333), 1 female; Hongkong, China (Koebele, No. 1333), 3 females.

("1333. First found with *Synonymyha grandis* upon *Bambusa* infested with *Oregma bambusae* Buckton, at Hongkong, China. Again at Kowloon, Hongkong, Nov. 3, 1895, upon *Celtis* infested with a whitish cottony covered aphid. Also upon *Stillingia sebifera* infested with aphids and leafhopper. Nov. 6, 1895, again one specimen with *Synonymyha grandis* upon aphids on bamboo.")

CYPHOCARIA CROTCII

Cyphocaria duvaucelii (Mulsant)

China (George Compere), 1 female.

ARTEMIS MULSANT

Artemis circumusta Mulsant (Plate II, Fig. 17)

China (Koebele, No. 1338), 1 female, belonging to the var. *mandarina* Mulsant.

("1338. One specimen only, upon mulberry badly infested with a small *Psylla*, causing the leaves to curl up. Feb. 22-Mar. 6, 1900, several specimens found at above place, Kowloon, sent to Mr. Craw and liberated (2) at Honolulu.")

LEMNIA MULSANT

Lemnia biplagiata (Schönherr)

China (Koebele, No. 1317), 2 females; Hongkong, China (Koebele, No. 1317), 4 males, 1 female.

("1317. On pine infested with *Lachnus*, Hongkong, Macao, no doubt feeding upon the plant lice. More numerous upon trees infested with *Lecanium* and *Pulvinaria* at Kowloon, Nov. 8, 1895. At Kowloon, many more upon *Celtis* and trees around same. On orange and *Psidium* infested with *Lecanium* and *Pulvinaria*. Common at Kowloon, Nov. 18, 1895.")

Lemnia biplagiata personata (Weise)

Formosa (Koebele, No. 1345), 1 male.

("1345. Common upon aphids on rose, *Lachnus* on pine, on tea plants, etc. many specimens. Tamsui, Formosa, Dec. 6, 1895, chiefly on rose aphids.")

Lemnia saucia calypso (Mulsant)

China (Koebele, No. 1346), 4 males.

("1346. Resembling the foregoing [1345] but always with the red spot much smaller. Common at Swatow, hibernating on various trees and shrubs, Dec. 11, 1895. Honolulu, March 4, 1896, many specimens on aphids on orange at Mr. Jordan's.")

COELOPHORA MULSANT

The type of *Coelophora* is *inaequalis* Fabricius, as designated by Crotch in 1874. Before proceeding with the enumeration of the species of *Coelophora* contained in the Koebele collection it is well to call attention to the numerous forms that have been assigned by various authors to *inaequalis* and the difficulties involved in determining the proper use of the names proposed by Fabricius and Thunberg.

According to the nomenclature used by most modern authors *inaequalis* includes a complex of forms ranging from the Australian continent on the south, northward through the Malayan Archipelago to the Philippines. Crotch records *inaequalis* from Japan but I have not seen it or anything similar either from Japan or the coast of China. As the distribution is mainly insular it is probable that any structural difference that may exist would not prove to be exactly or completely intergradant from one island group to the next.

On comparing the male genitalia of the Australian and Philippine forms assigned by authors to *inaequalis* one will find a notable difference. Moreover the genitalia of allied forms from Java, Borneo, and other islands differ from the Australian or Philippine forms although closer to the latter. The material that I have been able to study is too inadequate to elucidate the problems, except partially, but I venture the opinion that the Australian and Philippine forms should be segregated at least as subspecies. The application of the names proposed by early authors, however, remains to be settled. The habitat of species described by Fabricius and Thunberg is apt to be vaguely indicated at the best, and the proper application of names on the basis of distribution may be doubtful. I believe, however, that the name *inaequalis* is applicable to the Australian species and that no name is available for the Philippine form.

A word is necessary about the color phases of *inaequalis* and allied species. In 1922 (Proc. Haw. Ent. Soc. 5, pp. 121-133) I showed that the Australian *inaequalis* has three phases, one of which, a nearly all black form, is a Mendelian recessive to the other two. This black form is the *Coelophora mastersi* of Blackburn. The other variant is a form showing nine small black spots or dots on the elytra. This is the *C. 9-maculata* (Fabricius). I have not seen any color phases corresponding to these from the Philippines, but in the Malayan Archipelago there seems to be a multiplicity of forms.

Coelophora inaequalis (Fabricius)

Australia (Koebele, No. 17 and 1017), 3; Sydney, New South Wales (Koebele), 1; Brisbane, Queensland (Koebele), 1; Kuranda, Queensland (Koebele), 1; Bundaberg, Queensland (Koebele), 8, one marked Oct. 1904; Cairns, Queensland (Koebele), 6; Hawaii (Koebele, No. 1017), 2.

("1017. Three specimens found upon lemon trees at Parramatta, New South Wales, Oct. 28-30, 1892, probably feeding upon aphids in larval state. Feb. 1, 1893. Found everywhere in places visited up to date and always upon Aphidae. At Too-woomba, on woolly aphids (?), very numerous. At Clarence River upon aphids on orange. Parasite on larva bred. Johnston River, etc. Also New Caledonia and Fiji.

"Common all over and around Honolulu, March 4, 1896. Bred parasites, May 6, 1896, Honolulu. Mr. Wait informs me that this parasite has been observed long

on the Islands upon *C. abdominalis* and is very numerous on that species in North Kona, Hawaii. Has spread on all the islands up to the highest mountains within two years, 1894–1896.

"July 23, 1896, at Kilauea, Kauai, millions on aphids infesting sugar cane, which they cleaned out in three weeks or so.

"Aug. 3, 1896. Mr. John Kidwell, Manoa Valley, reports that owing to this beetle, the disease so prevalent on the taro had entirely disappeared, although the aphids were still present.

"September–October, 1896. Found badly parasitized on every Island visited and especially so at Kona and Lahaina.")

***Coelophora inaequalis*, var. *9-maculata* (Fabricius)**

Australia (Koebele, No. 1158), 3; Toowoomba, Queensland (Koebele, No. 95), 1; Bundaberg, Queensland (Koebele), 1; Cairns, Queensland (Koebele, No. 1158), 18.

("1095. One specimen only at Toowoomba upon orange, April 9, 1892. At Bundaberg, Queensland, June 1904, on sugar cane, likely upon aphids. Also again at Kuranda, various plants, Hambleton, Mulgrave, Cairns, Queensland, upon sugar cane. July, September, 1904, a considerable number sent to Honolulu. September to October, 1904, at Bundaberg, rare on sugar cane and various trees, while beating, and few obtained to send to Honolulu."

"1158. Several specimens on various trees and shrubs at Kuranda, Queensland, July 1894. Had been observed feeding upon orange aphids on a citron tree.

"Aug. 6, 1894. Found male in copula with female No. 1160, on orange infested with aphids. Also noticed them copulating with *Coccinella repanda* [as *Coelophora inaequalis* was called by Koebele], and vice-versa.

"Three specimens mounted of typical form, formerly under *C. repanda*, collected at Sydney, New South Wales, January 1905.")

***Coelophora inaequalis*, var. *mastersi* Blackburn**

Cairns, Queensland (Koebele, No. 1160), 5.

("1160. One specimen on orange at Kameranga, Queensland, apparently feeding upon aphids. Aug. 6, 1894, found female in copula with male No. 1158, upon orange tree.")

***Coelophora inaequalis comperei* new subspecies**

Similar to typical *inaequalis* from Australia and commonly referred to it, but differing in details of coloration, sculpture and male genitalia.

Form as in *inaequalis*, but the insect, somehow, gives the impression of being more compact, with a thicker, or heavier, integument. Prothorax and elytra less shining, as finely punctured as in *inaequalis*, but having fine pellucid dots at the base and along outer margin of elytra and others in a line running back from the outer side of the callus. These dots resemble coarse punctures and are hardly indicated in *inaequalis*. Ground color a dull flavous or reddish as in *inaequalis*, including that of head, underparts and legs. (In *inaequalis* the femora, coxae and greater part of sternum and venter are black.) Base of pronotum black, the black area much wider in middle, reaching from base to apical fourth, and deeply and generally broadly emarginate in median line almost to the base. On each side the black area is comparatively narrow, either uniformly or with a dilation laterad. Markings of elytra as in *inaequalis* except that the outer margin is not blackened and the subapical spot and outer submedian spot do not reach the outer margin. Suture with a black vitta as in *inaequalis*, slightly dilated between the middle and scutellum and rhombically dilated behind. Basal spot with its outer margin resting on the callus, rounded or oval, with a broad stalk extending backward between the

submedian spots and thereby sometimes more or less joined to either one or both of these spots. Subapical spot less irregular in shape than in *inaequalis* and having the shape of a solid B, with the two lobes more or less well indicated and equal. (In *inaequalis* the inner lobe of the B is much larger than the outer lobe.) Length, 4.5 to 5.4 mm., width, 3.7 to 4.7 mm.

Male genitalia similar to those of *inaequalis* but differing as follows: Median lobe of tegmen distinctly broader, about four times longer than wide. (In *inaequalis* about five times longer than wide.) Apical half of ventral surface with a median keel or carina, on each side of which the surface is slightly concave. This apical bilaterally concave area bounded basad on each side by a carina curving off from the siphonal orifice to the lateral margin of the lobe. (In *inaequalis* these carinae turn backward and merge with the median carina near the beginning of the apical fifth of the lobe.) Tubercle bordering apex of siphonal orifice on ventral surface of lobe weakly developed and not distinctly protruding as seen in some allied forms. (In *inaequalis* this tubercle appears triangular in direct view and moderately projecting in profile.) Siphon practically as in *inaequalis*, with two marginal scallops on each side just beyond the middle, and the apical part abruptly bent a little after the beginning of the last fourth, beyond which it tapers rapidly to a tenuous point. Ventral surface of siphon not or hardly bulging opposite the scalloped margin as in some forms of the *inaequalis* group.

Described from 8 specimens (holotype ♂ and paratypes) collected at Los Baños, Luzon, Philippine Islands, July 1916 (F. X. Williams), and 2 specimens (paratypes) from Manila (Geo. Compere) in the Koebele collection.

Types in collection of the Hawaiian Sugar Planters' Experiment Station.

Coelophora veranioides Blackburn

Windsor, New South Wales (Lea), 3; Mt. Victoria, Sydney, New South Wales (Koebele), 1; Harwood Island, New South Wales (Koebele, No. 97 and 97A), 3; Toowoomba, Queensland (Koebele, No. 97), 1; Bundaberg, Queensland (Koebele), 4.

("1097. Two specimens saved; one Harwood, Clarence River, New South Wales, Jan. 1, 1892, another at Toowoomba, Queensland, on orange.")

MICROCARIA CROTCH

Probably *Anisolemmia* Crotch, 1874, is a synonym of *Microcaria* Crotch, 1871. I have not been able to see the type species (*A. complicata* Crotch) of *Anisolemmia* but I have examined *A. ceramensis* Crotch and believe that it is congeneric with *M. mulsanti* (Montrouzier). *Coelophora jansoni* Crotch is also congeneric. Rye in 1873 (Zool. Rec. 8, p. 329) selected *mulsanti* as the type of *Microcaria*.

Microcaria mulsanti (Montrouzier)

New Caledonia (Koebele, No. 2416), 4.

Microcaria jansoni (Crotch)

Cairns, Queensland (Koebele), 3; Geraldton, Johnston River, Queensland (Koebele, No. 1182), 4.

("1182. At Geraldton, Queensland, Aug. 10-15, 1894, upon hibiscus tree, badly infested with a psyllid, five specimens of a coccinellid feeding upon those insects.")

BOTHROCALVIA CROTCH

Coelophora pupillata (Schönherr) falls in *Bothrocalvia* and it is almost identical in the male genitalia with *B. albolineata* (Schönherr).

Bothrocalvia albolineata (Schönherr)

China (Koebele, No. 1307), 54; Hongkong, China (Koebele, No. 1307), 9; Formosa (Koebele, No. 1307), 3.

("1307. Common upon *Pinus sinensis* at Hongkong, China, Oct. 19, 1895. As yet I have no idea upon what this may live.

"Pines infested with *Lachnus*, Macao, Oct. 23, 1895. Bred from larva feeding upon *Lachnus*. Always on *Lachnus* on pine.

"At Tamsui, Formosa, upon *Pinus sinensis* infested with *Lachnus*, all stages.")

Bothrocalvia pupillata (Schönherr)

China (Koebele, No. 1318), 2; Hongkong, China (Koebele, No. 1318), 7.

("1318. One specimen near Hongkong upon *Camellia*, which appeared to be clean, Oct. 26, 1895. Many at Kowloon upon *Stillingia sebifera*, *Celtis sinensis*, *Psidium* sp., etc., Nov. 8, 1895. About 1,000 specimens collected on *Celtis* and trees adjoining these. Larvae still present, pupa found on bark of branch.

"March 4, 1896, two specimens upon orange infested with aphids, Honolulu. Aug. 12, 1896, one specimen in nursery upon cashew, *Anacardium occidentale*. Again at same place, Nov. 10, 1896.")

Bothrocalvia pupillata, var. **annectans** new var. (Plate II, Fig. 18)

Agreeing with typical *pupillata* except as follows: The two subsutural black spots on each elytron instead of being separately ocellated with a pale ring are enclosed within two pale vittae joined at their ends. The black spots are placed at the ends of this enclosure. The three submarginal spots on each elytron are separately ocellated as in *pupillata* but the pale rings are joined together by a pale vitta. This vitta runs tangent to inner margin of the ocellation of the middle spot, and hits the inner posterior edge of the subhumeral ocellation and the outer anterior edge of the subapical ocellation. These markings, therefore, are an almost exact combination of the black spots of *pupillata* and the vittate markings of *albolineata*. The male genitalia of *annectans* are exactly as in *pupillata* and differ hardly at all from those of *albolineata* except in having the paramera distinctly more slender and much more arched dorso-ventrally in the basal half.

Described from 4 specimens (holotype and paratypes) from China (Koebele, No. 1318). According to Koebele's field notes, the type locality is Hongkong and vicinity.

PHRYNOCARIA NEW GENUS

Having the characters of *Coelophora* Mulsant in general, but distinguished as follows: Frons comparatively narrow, at its narrowest part about, or even less than, one-third the total width of head, with the inner orbits of eyes strongly diverging anteriorly. The greater width of frons in *Coelophora* seems to be partly in consequence of the smaller size of the eyes and partly in consequence of the larger size of the whole head. The postantennal canthus of *Phrynocaria* is narrow and rather deep; in *Coelophora* it is much broader exteriorly and the emargination of the eye therefore appears to be shallower.

Median lobe of tegmen short and broad, not greatly longer than broad, strongly depressed above and beneath, strongly arcuate on the sides and roundish at apex, with a minute median nipple-like projection. Paramera well separated at their bases, slender, gently curved and somewhat surpassing apex of the median lobe. Siphon rather short, moderately slender, tapering to a fine point at apex, but not tenuous and not expanded between the middle and beginning of the apical third. It is also more chitinated, with much less evident separation of the component strands than in allied *Coelophorine* genera, and is armed near beginning of the apical third, or between that point and the middle, with a laminate projecting lobe on each side from the ventral wall. These lobes form more or less evident retrorse hooks, especially well chitinated and conspicuous in *congener*.

Genotype: *Coccinella congener* Schönherr.

Phrynocaria congener (Schönherr)

China (Koebele, No. 1327), 13; Hongkong, China (Koebele, No. 1327), 2.

("1327. Four specimens, Hongkong, China, Oct. 29, 1895; one male with sides of thorax white, on pine; the others on *Psidium* infested with *Lecanium* and *Pulvinaria*.")

Phrynocaria congener, var. approximans (Crotch)

China (Koebele, No. 1331), 17.

I have thrown these together as forms of one species on account of the identical structure, including that of the genitalia. The median lobe of the tegmen in this species is shorter and broader than in *gratiosa* and the siphos have much better developed retrorse hooks which are situated at the beginning of the apical third.

("1331. Resembling 1318, yet smaller, sides of thorax white and but four black spots on elytra. Five specimens, Kowloon, Hongkong, Nov. 3, 1895, with 1318 upon *Celtis* chiefly infested with an aphid. Nov. 8, 1895, several more upon *Celtis* and pine at Kowloon. Food not yet observed. On orange infested with *Lecanium* and *Pulvinaria*, Kowloon, Nov. 18, 1895.")

Phrynocaria gratiosa (Mulsant)

In this species the median lobe of the tegmen is about or nearly twice as long as wide, and the hooks of the siphos are small and membranous and situated about half-way between the middle and the beginning of the last third.

In 1894 Mr. Blackburn (Trans. Roy. Soc. Aust. 18, p. 338-339) reported on a series of specimens of this species sent to him by Mr. Koebele and remarked that, if he was right in associating the remarkable series of variations, the species was one of the most variable of the Coccinellidae. He then describes, without naming them, some nine variations, including the typical form. It seems desirable to have names for the major types of variation and I consequently name those found in the Koebele collection. This is the more important as some of them look like distinct species, and may actually be distinct, although on account of the identical genitalia I am persuaded to place them together here as varieties. As Mr. Koebele had included all these variations under his No. 1139, he had evidently reached the same conclusion by field observations.

("1139. Numerous at Brisbane upon various trees in larva, pupa and imago state, feeding upon *Lecanium depressum*. A very variable species. Many mounted, others sent to Honolulu. Many bred from light colored pupae. A few specimens at Toowoomba, April 28-30, 1897, upon orange, no doubt feeding upon *Lecanium*. Common near Bundaberg, Queensland, during early spring near sea-shore, but not breeding, September and October, 1904.")

Typical gratiosa (Plate II, Fig. 19)

Bundaberg, Queensland (Koebele), 1 female.

Elytra black, with a broad transverse band at base of each and large mark at apex, yellow. The entire margin of each elytron is black although very narrowly so across the base. The common median black band extends triangularly forward to the scutellum. Pronotum black, except narrow anterior margin, and a large oval mark on each side, not quite reaching base, which are yellow.

Phrynocaria gratiosa, var. flavoguttata new var. (Plate II, Fig. 20)

Bundaberg, Queensland (Koebele), 6 (holotype and paratypes); Toowoomba, Queensland (Koebele, No. 92), 1 (paratype); Brisbane (Koebele, No. 1139), 4 (paratypes), one of these also labeled "Toowoomba."

Similar to the typical form, but the basal pale band divided into two marks by an extension of the black over the callus to the base, and the median black band enclosing two large pale marks on each elytron. The black thus forms a reticulate pattern, with five large pale marks on each elytron. Mark 1 between callus and suture at base, subquadrate, with inner margin oblique. Mark 2 on outer side of callus at base paralleling the outer margin for nearly one-third the length of the elytron, and emarginate on inner side by the black callus. It is thus subreniform or like a thick inverted comma. Mark 3 triangular and 4 subquadrate, forming with those of other elytron a transverse series just behind the middle. Mark 5 apical and practically as in typical form. Minor variations of this general type are as follows:

1. Marks 3 and 4 confluent to a more or less degree.
2. Mark 4 connected with 5 at their outer margin by a slender pale line paralleling the elytral margin. This variation sometimes combined with the preceding.
3. Marks 1 and 2 confluent as in typical *gratiosa*, or with an isolated small black spot left on callus. This may occur in conjunction with the union of marks 3 and 4, so that each elytron is pale, margined all around with black and with two transverse irregular black bands at about two-fifths and two-thirds of the length.

The pale coloration in *flavoguttata* varies from reddish yellow to creamy white. The pronotal markings typically as in *gratiosa*. In the minor variations the black area may cover about three-fourths of the length, leaving a much broader pale anterior border, and it may also become deeply emarginated by the pale color in the median line nearly to the base.

***Phrynocaria gratiosa*, var. *palens* new var. (Plate II, Fig. 21)**

Brisbane, Queensland (Koebele, No. 1139), 2 (holotype and paratype).

This differs from *flavoguttata* by the entire lack of the black reticulate pattern, the margins only remaining black, with the sutural border dilated between the middle and the scutellum. Pale color of pronotum and elytra creamy white, or white marked with testaceous. In the type each elytron has a large testaceous brown cloud across the middle, not quite reaching the black marginal border and enclosing an oval creamy white mark between itself and the black suture just behind the dilation of the latter. The base with a broad transverse band of creamy white, the apex with a large mark of the same color, the two being joined next to the outer margin by a white line lying between black and brown areas.

In the other specimen the brown cloud extends forward to the base and encloses a broadly oval creamy white mark between itself and the black sutural dilation at the base. The oval creamy white spot next to the suture just behind the middle is present but smaller than in the type. The apical creamy white mark is also smaller. The brown cloud is separated from the black border of outer margin by a narrow pale vitta from base to apex, this vitta being whitish, marked with testaceous brown.

The black area on pronotum as in typical *gratiosa* except that its anterior margin may have a small triangular median emargination and a small whitish spot is present in the median line just before the base.

***Phrynocaria gratiosa*, var. *nigrocincta* new var. (Plate II, Fig. 22)**

Bundaberg, Queensland (Koebele), 10 (holotype and paratypes); Toowoomba, Queensland (Koebele), 1 (paratype).

Differs from the preceding varieties in having the disk of pronotum and of each elytron a unicolorous tawny (dull brownish yellow). Pronotum narrowly margined with black on the outer margin from the middle to the base. The basal margin also is narrowly margined with black but this black portion is mostly normally covered by the elytra. Scutellum black. Basal and sutural margin of the elytron very narrowly margined with black, the outer margin with a rather narrow black border, narrowed behind, precisely as in preceding varieties. There is sometimes a black dot on the callus (present in six out of eleven specimens, but absent in holotype), and in one specimen there is a black dot at the anterior third and barely closer to the suture than to the outer margin.

This is the var. G of Blackburn and very likely the insect recorded by Crotch from Australia as *Coelophora versipellis* Mulsant, but *versipellis* is a genuine *Coelophora*, closely allied to *C. inaequalis* (Fabricius).

Phrynocaria gratiosa, var. **nigrovittata** (Blackburn) (Plate II, Fig. 23)

Bundaberg, Queensland (Koebele), 5; Toowoomba, Queensland (Koebele, No. 1139), 1; Brisbane, Queensland (Koebele, No. 1139), 2; Cairns, Queensland (Koebele), 2.

In this variety each elytron has the sutural and outer margin bordered with black and three black vittae on the disk. The basal margin of the elytron is also narrowly margined with black. The sutural border is uniform in width and is abbreviated just behind the scutellum. The middle vitta extends from the callus to apical seventh but is usually interrupted subapically. The remaining portion of this vitta is broadly twice indented on its inner margin, or even interrupted, in which case the vitta is broken into four spots. The outer vitta joins with the middle one at the callus. The inner vitta parallels the suture and extends to the beginning of the apical third. The pronotum has an irregular black border across the base and on the posterior half of the lateral margins. On the anterior middle of the disk is a black mark shaped much like a spread butterfly, facing forward.

This was described by Mr. Blackburn as a distinct species in 1895 (Trans. Roy. Soc. So. Aust. 19, p. 237) and certainly looks distinct, but the male genitalia are precisely as in the preceding varieties.

Phrynocaria gratiosa, var. **koebelei** new var. (Plate II, Fig. 24)

Bundaberg, Queensland (Koebele), 9 (holotype and paratypes); Cairns, Queensland (Koebele), 2 (paratypes); Brisbane, Queensland (Koebele, No. 1139), 1 (paratype); Australia (Koebele), 1 (paratype).

This differs from all the preceding varieties in lacking the black border on outer margin of elytra. The sutural border is somewhat dilated anteriorly and more or less distinctly abbreviated just behind scutellum. It has also a slight punctiform dilation close to the sutural angle. The basal and outer margins are either entirely pale or extremely narrowly edged with black on the marginal bead (as in holotype). Disk of each elytron with four (or five) rather large black marks. Marks 1, 2 and 4 forming an interrupted vitta from callus obliquely to apical fourth of length and inner third of width. 1 + 2 extends to the middle of length and is dilated behind the callus on inner side. Rarely it is divided into two spots: 1 small, on the callus, and 2 much larger behind. Mark 3 lying half way between the portion 2 or 1 + 2 and the outer margin of elytron. Mark 4 somewhat obliquely oval, its inner posterior margin more or less approaching the sutural border. Mark 5 quadrate or triangular, often larger than either 3 or 4 (larger and triangular in holotype) and lying between mark 4 and outer margin and usually about equally distant from each. Ground color either unicolorous tawny (holotype), or sometimes with five paler areas corresponding more or less exactly in position and form to the pale guttate marks of *flavoguttata*. In such cases the black marks are bridged together by the tawny ground color to form a more or less evident reticulate pattern. In one paratype the ground color is very pale tawny and the five paler areas almost creamy white. Pronotal pattern similar to that of typical *gratiosa*, or *flavoguttata*, but with apical pale border broader and with a pale mark (or two dots) in the middle before the base. The latter mark varies greatly in size and sometimes extends laterally to isolate a black butterfly-shaped mark as in *nigrovittata*.

This variety seems to be derived from *flavoguttata* by the loss of the marginal border and by having the black reticulate pattern interrupted in such a manner that only a small portion of the longitudinal and transverse bars remain.

EOCARIA NEW GENUS

Form broadly oval, with coloration scheme as in *Vibidia*. Pronotum with the outer margins gently arcuate and convergent in front, the posterior corners subangulate. Thoracic epipleura

with a distinct oval fovea. Elytra somewhat broader than base of pronotum, moderately explanate on outer margin, the epipleura rather less wide than in *Coelophora*, almost horizontal and very slightly foveate to receive apex of hind femora. Prosternal process bicarinate, the carinae parallel and extending about three-fourths of the distance to the anterior margin of segment. Mesosternum deeply and angularly emarginate in the middle. Metasternum transverse between the middle coxae. Coxal lines of first ventrite curving backward and outward from the base and becoming confused with the posterior margin of the segment. Frons moderately narrow, the inner orbits of eyes distinctly convergent behind. Antennae rather long, reaching to the front coxae. Joints 3 to 8 slender, much longer than thick, except 7 and 8 which are only slightly longer than thick. Joints 9 to 11 forming a club, with 9 and 10 both much wider at apex than at base, 9 slightly longer than wide and 10 about one and one-third times wider than 9 and barely wider than long. Joint 11 broadly oval, about one and one-fourth times longer than wide and truncate at apex. Puncturation of pronotum rather fine and close, that of elytra a little sparser and rather distinctly unequal.

Median lobe of tegmen as seen from above very narrow at base, abruptly bent downward near the beginning of the second fifth and at the same point beginning to expand into an elongate oval body, which is narrowed into a slender upturned point at apex. As seen from the side the median lobe is strongly compressed beneath in the constricted basal part and depressed on apical half both above and beneath. Paramera slender throughout, almost perfectly straight, reaching to the apex of median lobe and strongly ciliate at apex and on outer margin as far as the middle. Siphon short and rather stout, moderately swollen from middle to beginning of the apical third, with evident separation of the dorsal strands, and armed beneath near apex of swelling with a pair of small hook-like processes from the ventral strands. Just beyond the swelling the dorsal strands are twisted one over the other and rapidly taper into a slender part which is slightly constricted close to apex. The short portion beyond the constriction more membranous and bent slightly downward.

From *Coelophora* this genus differs in the broadly oval form, with the posterior angles of pronotum much more evident, the frons narrower (but not so narrow as in *Phrynocaria*), and the sternum much more convex. With *Propylea* it agrees in general form and structure, but the prothoracic foveae are rather more evident, the frons decidedly narrower, the elytral puncturation distinctly unequal and the coloration and male genitalia very different.

Genotype: *Eocaria muiri* n. sp.

Eocaria muiri new species (Plate II, Fig. 25)

Japan (Koebele, No. 1237), 1 ♂, 1 ♀ (holotype ♂ and allotype)*. Also two paratypes collected in Japan (Okitsu, June, 1913, and Karuizawa, Aug. 1913) by Frederick Muir and in the collection of the Hawaiian Sugar Planters' Experiment Station. Also the following paratypes in U. S. National Museum: 1 ♂, 1 ♀, Karuizawa Mts., Japan; 2 ♀, Kyoto, Japan; 1 ♂, Shin Kai Si, Mt. Omei, 4500 ft., Szechwan, China, Aug. 7, 1929 (D. C. Graham); and 1 ♂, 2 ♀, Suifu, Szechwan, China, 1922, and June 21, 1928 (D. C. Graham).

Very similar in size, coloration and markings to *Vibidia 12-guttata* (Poda), with which species it has been found confused in collections, but somewhat more broadly oval, the pronotum with four white spots across the base instead of two, and the two outermost spots on each elytron widely separated from the margin.

Rich orange brown or fawn color above and beneath, with the palpi, antennae and legs about concolorous. Mesepimera, anterior corners of pronotum, and frons, more or less, creamy white. Pronotum also with four creamy white spots at the base. The two inner ones placed on either side of middle, diverging in front and usually not quite touching the basal margin. The other two broader, either quadrate or rounded, and placed on the basal margin just within the posterior angles. Each elytron with six oval, or broadly oval creamy white spots, of nearly

* For Koebele's field note, see *Vibidia 12-guttata* (Poda), with which this species was confused.

the same size and arrangement as in *Vibidia 12-guttata* (Poda) except that the two outer spots (3 and 5) are well removed from the lateral margin. Spot 6 usually more or less quadrate and transverse, the others having their longer axis parallel to the suture. Spots 1, 4 and 6 equidistant from the suture, and 2 about twice as far removed. Spot 3 placed behind and slightly exterior to the callus. Spots 2 and 4 are each somewhat posterior to 3 and 5 respectively. The channel paralleling the lateral margin is more or less whitish, especially at the basal and apical angle. At the latter angle it is often dilated into a well defined creamy white spot which may be more or less confluent with spot 6. Male genitalia as defined under the generic heading.

Length of Japanese specimens, 4-5 mm.; of the Szechwan specimens, 5.25-5.5 mm.

GYROCARIA NEW GENUS

Form subhemispherical, or very broadly oval, the elytra at base only slightly wider than pronotum. Sides of pronotum gently rounded, convergent in front, and very finely margined. Head rather large, the frons broad, the inner orbits of eyes subparallel. Canthus of eye rather deep and narrow. Clypeal margin slightly emarginate. Antennae rather short, not surpassing anterior margin of front coxae. Antennal joints 3 to 7 very slightly longer than thick, 8 as long as wide, 9 and 10 transverse, 11 wider than long, obliquely rounded at apex. Joints 8 to 11 forming a compact club. Epipleura of prothorax shallowly foveate on inner margin anteriorly, the fovea covering about one-fifth to nearly one-third of the length. Prosternal process rather short and broad, only slightly surpassing front coxae, with two submedian longitudinal carinae, which extend considerably in front of the coxal cavities. Mesosternum broadly and rather shallowly emarginate medially, its anterior border sharply declivous. Metasternum truncate and rather broad between middle coxae. Coxal lines of first ventrite curving outward to hind margin of segment, the oblique line present but indistinct. Elytra subexplanate and strongly margined at sides, the epipleura rather broad, descending externally and subfoveate to receive apex of hind femora. Puncturation fine but rather strong, more or less coarser on explanate outer margin of the elytra, and sometimes slightly unequal on their disks. Fifth ventrite in male strongly and very broadly emarginate at apex, the sixth much less broadly emarginate, but exposing a very short seventh segment. Pronotum black, the sides broadly white or pale yellow. Frons in female black, pale in male.

Median lobe of the tegmen short, depressed, deeply emarginate at apex, the process on each side of emargination acute (the structure resembling an old-fashioned bootjack and recalling condition found in *Synharmonia*, but the lobe much shorter, not turned upward at apex and much more deeply emarginate). Paramera short and straight, equalling the length of median lobe and well separated at base. Siphon short, rather stout, and rather abruptly tapering in the apical tenth into a very fine membranous point.

Genotype: *Coclophora guttata* Blackburn.

Gyrocara guttata (Blackburn) (Plate II, Fig. 26)

Australia (Koebele, No. 1159), 5; Cairns, Queensland (Koebele, No. 1159), 7 specimens, one of these also labeled "Kuranda, Geraldton."

("1159. One specimen at Kuranda, Queensland, on orange. Common at Geraldton, Queensland, and breeding upon psyllids infesting hibiscus tree, Aug. 10-15, 1894.")

AIolocARIA CROTHI

Aiolocaria mirabilis (Motschulsky)

Japan (Koebele, No. 1300), 5 specimens.

("1300. First observed at Gifu, Japan, beginning of April 1895, and said by Mr. Nawa to feed upon the eggs and larvae of a *Chrysomela* infesting willows. Here a number of specimens were found and forwarded to Honolulu. Eggs were present upon the trees in large numbers and some brought to Yokohama and placed

upon hibiscus infested with aphids, were lost sight of, evidently they will not feed on this. At the end of June, at Nikko, the beetle again was met with and feeding upon a chrysomelid larva infesting walnut; many eggs were noticed there as well. Without any doubt this would feed upon the potato beetle in America. Many eggs were found on walnut, where no trace of the chrysomelid was noticed. Still breeding during July upon coleopterous larvae on walnut. Noticed one larva running around on tree, upon which no more food was present, and meeting with a large syrphid larva it devoured the same. A chrysomelid was numerous and destructive on *Alnus incana* and none of these beetles were ever observed, but fed with these larvae in confinement they would devour the same. Mature insect was also observed upon willow, at Nikko, infested with a small blue chrysomelid, but the larva was not found.")

SYNONYCHA MULSANT

Synonymcha grandis (Thunberg)

Ceylon (Koebele, No. 1200), 1; China (Koebele, No. 1200), 2; Hongkong, China (Koebele, No. 1200), 12; and 7 specimens without data.

("1200. Three specimens near Kandy, Ceylon, December 1894, feeding upon a large aphid infesting the bamboo plants. Found more numerous during January and February 1895, and always feeding on the large aphid, which according to Mr. Green is *Oregma bambusae* Buckton. Noticed eggs during January, yet these never hatched and no larvae were observed.

"Oct. 15, 1895, common at Hongkong, upon same aphid, in larva, pupa and imago stage. Swatow, China, December 1895.

"One apparently fresh specimen upon orange at Jordan's place [Honolulu] no doubt feeding upon aphids, May 6, 1896.

"Jan. 17, 1897, mounted parasites (*Homalotylus*) and secondary parasites from larvae collected at Hongkong, China, marked No. 1200.

"Feb. 28, 1900, a few aphids on bamboo at Hongkong but beetle not breeding yet, although present.")

CHEILOMENES CHEVROLAT

The type of *Cheilomenes*, as selected by Crotch, 1874, is *Coccinella lunata* Fabricius. The same species must be taken as the type of *Cydonia* Mulsant, so that the latter name falls in synonymy.

Cheilomenes sulphurea (Olivier)

Ukani, German East Africa, 1 male.

This is distinct from the South African *C. lunata* (Fab.), not only in the markings but also in the male genitalia.

MENOCHILUS NEW NAME

The genus *Cheilomenes* Mulsant and other authors, with *Coccinella sex-maculata* Fabricius as type, needs a new name and may be called *Menochilus*.

Menochilus sex-maculatus (Fabricius)

Ceylon (Koebele, No. 1217), 2; Formosa (Koebele, No. 1347), 1; Manila, Luzon (Geo. Compere), 1 specimen.

("1217. Three specimens brought to me and said to have been collected with No. 1209 amongst grass at Kandy, Ceylon, Jan. 10, 1895.")

"1347. A few specimens only, hibernating on screw palm chiefly, Swatow China, Dec. 11, 1895. Also at Tamsui, Formosa, December 1895.")

Menochilus quadriplagiatus (Schönherr)

China (Koebele, No. 1316), 19; Hongkong, China (Koebele, No. 1316 and 1338), 24.

("1316. One pair in copula at Macao, China, Oct. 24, 1895, upon *Acacia* (?) infested with *Dactylopius*. Nov. 1, 1895, very numerous at Botanic Gardens, Hongkong, feeding upon *Dactylopius* infesting a creeping *Ficus*, in larva, pupa and imago states, on aphids infesting *Hemerocallis*, in all stages; upon pine and various other trees. Common on weeds infested with aphids; in all stages at Kowloon, Nov. 18, 1895. Swatow, Dec. 11, 1895 and Kowloon, Dec. 13, 1895, common in all stages upon aphids on egg plant. From pupa likely of this insect, a parasite was bred and is marked 1316. Tetrastichine.")

For No. 1338 see *Artemis circumusta* Mulsant, with which Koebele evidently confused this species.

HALYZIA MULSANT

Halyzia sedecim-guttata (Linnaeus)

Waldkirch, Baden, Germany (Koebele), 5; Pelussin, France, August 1900 (Koebele ?), 1 specimen.

NEOHALYZIA CROTCII

Neohalyzia perroudi (Mulsant)

Mexico (Koebele, No. 1685), 1 female.

("1685. But two specimens, while beating at base of Popocatepetl, Mexico, May 1897, about 10,000 ft. elevation. A very peculiar pale form and resembling *Epilachna* except in want of pubescence.")

VIBIDIA MULSANT

Vibidia duodecim-guttata (Poda)

Japan (Koebele, No. 1237), 2 females.

("1237. Few at Miyanoshta and more at Atami, April 1895, upon various trees and not upon any particular food. Some sent to Honolulu. At Yokohama in larva state upon alder infested with mildew.")

PSYLLOBORA CHEVROLAT

As *Coccinella 20-maculata* Say, selected by Crotch, 1874, as genotype of *Psyllobora* was not originally included by Chevrolat, and hence not a valid type, I herewith select *Coccinella lineola* Fabricius, as the type of *Psyllobora*. *Thea* Mulsant is here included.

Psyllobora luctuosa Mulsant

Mexico (Koebele, No. 1648), 13; Guadalupe, D. F., Mexico, Nov. 1907 (Koebele), 2.

("1648. One specimen, beating at Oaxaca, Mexico, August 20, 1897. Very common on Popocatepetl, Mexico. Many mounted.")

Psyllobora koebelei Nunenmacher (Plate II, Fig. 27)

Arizona (Koebele, No. 2426), 1 male, 1 female.

I have labeled these paratypes as they are cited in the original description. According to Nunenmacher the specimens were collected at Nogales, Santa Cruz County, in June 1902.

Psyllobora parvinotata Casey

Florida, 1 female.

I regard *P. pallidicola* Blatchley almost unquestionably as the same species as *P. parvinotata* Casey, after comparison of specimens collected by Blatchley at Dunedin, Florida, with Casey's type. The elytral pattern proved identical and the Blatchley specimens (also the specimen cited above) differed only in lacking the pronotal marks which are well developed in Casey's type.

Psyllobora viginti-maculata (Say)

West Point, Nebraska, May 1888, 1.

Psyllobora borealis Casey

Oregon (Koebele), 5; Easton, Washington (Koebele), 2.

Psyllobora taedata Leconte

Alameda, California, April (Koebele), 1; Oregon (Koebele), 1.

Psyllobora deficiens Casey

Santa Cruz Mountains, California (Koebele), 1.

Psyllobora renifera Casey

Sonora, Mexico (Koebele, No. 1679), 4; Arizona (Koebele, No. 2418), 4; Argus Mountains, California, April 1891 (Koebele), 4; Panamint Valley, California, April 1891 (Koebele), 1.

("1679. Five specimens, beating at Hermosillo and Guaymas, Sonora, Mexico, April 1897. This insect [considered by Koebele to be the same as *P. taedata*] lives upon mildew on grape, rose, apple, etc.")

Psyllobora juvenca new species (Plate II, Fig. 28)

Similar to *P. renifera* Casey, but spots 1 and 3 free (1 sometimes joined laterally with 2), the others joined to form an irregular-shaped longitudinal mark, broadly incised at one-third and near the middle and nodosely enlarged on each side at two-thirds. Differs from *P. taedata* Lec. and *P. 20-maculata* (Say) in having dark markings of elytra distant from lateral margin. In respect to male genitalia similar to *P. renifera* Casey, but siphon slightly longer, the median lobe of tegmen less slender, compressed at base, slightly obtuse at apex which is only slightly upturned.

Described from 3 specimens, in collection of the Hawaiian Sugar Planters' Experiment Station, two of them belonging to the Koebele collection: 1 ♂ (holotype), Cuautla, Morelos, Mexico (Koebele); 1 paratype, Morelos (Koebele); and 1 paratype, El Potrero, Vera Cruz, Mexico (H. T. Osborn).

Psyllobora vigintiduo-punctata (Linnaeus)

Lyons, France, May 1897, 1 male; Waldkirch, Baden, Germany (Koebele), 5.

ILLEIS MULSANT

This genus is very distinct from other Halysiine genera in the structure of the antennae and maxillary palpi. The antennae are elongate, distinctly surpassing front coxae. Joints 3 to 5 considerably longer than wide, 7 also longer than wide,

but 6 and 8 shorter, sometimes much shorter, than the joints between which they are placed, and usually about as long as wide. Joints 9 to 11 forming a very loose club, 9 and 10 widening toward apex, where they are not greatly wider than the apex of joint 7. Apical joint, however, considerably wider than the preceding joints, and ovoid to subrotund in shape. Apical joint of maxillary palpi in the form of a transverse elongate-ellipsoid disk, attached to the preceding joint at the middle of its basal surface. Its outer surface is transversely creased.

Mulsant (1850) placed the species of *Illeis* under *Psyllobora* but separated them as a subgenus under the preoccupied name *Egleis* (p. 167). In the Appendix (p. 1026) he indicates that his key line, "A. Dernier article de la massue des antennes, ovoïde, court (*G. Egleis*)," should have preceded *P. galbula* on page 166 instead of *P. cincta* (p. 167), and substitutes *Illeis* for *Egleis*. In the body of the work he thus actually included *P. cincta* (Fab.) and *P. bistigmosa* Mulsant under *Egleis*, and in the appendix added *P. galbula* Mulsant, under the substitute name, *Illeis*. *Psyllobora galbula* Mulsant is here designated as the type of *Illeis*.

Weise, overlooking *Illeis*, proposed *Leptotheca* (Arch. Naturges, 64, p. 227, 1898) with *Psyllobora galbula* as the type.

***Illeis galbula* (Mulsant) (Plate II, Fig. 29)**

Australia (Koebele, No. 18, 481 and 1018), 13: Toowoomba, Queensland (Koebele), 1; Cairns, Queensland (Koebele), 6.

("481. A black and lemon-yellow coccinellid found only with the woolly aphid, numerous at Gordon, New South Wales, and also at Toowoomba, Queensland. Never met with this insect except feeding on above insect.

"1018. Found first at Gordon, N.S.W., upon apple trees infested with woolly aphid. Also at Toowoomba, Queensland, Nov. 9, 1892, upon same trees. At this place the eggs were found on trunk of an old Northern Spy. Many in spider's nest, also young larvae, no doubt feeding upon mildew.

"Nov. 14, one specimen upon orange at Parramatta, New South Wales.

"Nov. 19, placed large larva with aphid; this pupated two days later without feeding. Young placed with same food, yet got lost.

"One specimen at Harwood, New South Wales, and more on way to Richmond River, many upon orange. Baron River, Queensland, July 1894, one specimen.

"Jan. 2, 1899. Mr. Compere brought to me one dead specimen found in hospital grounds, Honolulu, under croton plant, upon which two living specimens were observed.")

***Illeis cincta* (Fabricius)**

Ceylon (Koebele, No. 1210), 6 females. A male with the same data was found in the collection of the Citrus Experiment Station.

("1210. One specimen by beating *Pittosporum* near Kandy, Ceylon, Jan. 1-7, 1895. One more running around tips of a plant, yet not any food could be observed.

"Sent to me by Mr. Green in all stages upon mulberry at Yokohama and elsewhere, Japan, on various trees, mulberry, *Alnus*, etc.

"At Hongkong, Oct. 19, on *Pyrus sinensis*, and very numerous upon *Stillingia sebifera*, where they breed upon mycelium.

"Common at Tamsui, Formosa, December 1895.")

***Illeis confusa* new species**

Like *I. cincta* (Fab.) and not distinguishable except by the male genitalia. Median lobe of tegmen acicular, strongly compressed and much deeper than wide in basal half, depressed, i.e., thin dorso-ventrally, and strongly curved upward at apex. In *cincta* (from Ceylon) this lobe is not distinctly curved upward at apex. It is less compressed at base so that the depth is hardly greater than width, and the apical part is compressed, instead of depressed, and ends very sharply as seen either from above or in profile. Paramera and siphon practically the same in both species, the paramera being gently arched, ciliate at apex, and about as long as median lobe of tegmen. The siphon is rather short, slender and simple.

China (Koebele, No. 1210), 4 males (holotype and paratypes), in Koebele collection; and the following paratypes: 2 males, 2 females, with same data as above, in U. S. National Museum; 7 specimens from Hongkong and 9 labeled West Australia, probably in error, collected by Geo. Compere, in collection of Citrus Experiment Station. The Koebele specimens are from Hongkong.

***Illeis koebelei* new species (Plate II, Fig. 30)**

Like *cincta* and *confusa* and distinguishable only by the male genitalia. Genitalia more similar to *cincta* than *confusa*, but median lobe of tegmen and paramera both shorter. Median lobe very acicular, compressed, sharp and only very slightly upturned at apex. As seen from the side the lobe at the base is much deeper than thick, but considerably less deep than in *confusa*. As seen from above it appears like a thin line. Paramera rather strongly curved at base and thicker in proportion to their length than in the other two species. Siphon similar except that it is more slender.

Japan (Koebele, No. 1210), 101 specimens (holotype ♂ and paratypes); Formosa (Koebele, No. 1210), 4 paratypes, in Koebele collection; and the following paratypes: 1, Japan (Koebele) in collection of Citrus Experiment Station; 3, Gifu, Japan (Y. Nawa), 2, Japan, in U. S. National Museum; also 13 from Szechwan, China (D. C. Graham) in U. S. National Museum, as follows: 8, Tseo Jia Geo, south of Suifu, 2,000-3,000 ft.; 1, Suifu; 1, Shin Kai Si, Mt. Omei, 4,500 ft.; 1, near Luting Kiao, between Fu Yao Linn Pass and Tatsienly, 5,000-6,000 ft.; 1, west of Yachow, 6,000 ft., and 1, west of Fu Lin, 4,000-8,500 ft.; also 2, Okitsu, Japan (F. Muir), in collection of Hawaiian Sugar Planters' Experiment Station.

***Illeis luzonica* new species**

This seems to be exactly like *amboinensis*, except in the male genitalia, as given in the table on page 61. The paramera are slender, straight, inserted very close together and ciliate at apex. The siphon is slightly more slender than in *amboinensis*. Length, 3.8 mm., width, 3 mm.

Described from 1 ♂ (holotype), Los Baños, Luzon, Philippine Islands, July 1916 (F. X. Williams), and 1 ♀ (paratype), from same locality, March-June, 1925 (Pemberton), in collection of the Hawaiian Sugar Planters' Experiment Station.

One female, Larat (F. Muir) appears to represent another undescribed species near *luzonica* and *amboinensis* but differs in the narrower, more oval form, and in having the pronotum less broad, with the side margins much less convergent anteriorly.

APPENDIX

TABLE OF GENERA ALLIED TO *HIPPODAMIA* AND *NAEMIA*

Body oval, more convex beneath than usual in *Coccinellinae*, head more exposed, the eyes generally fully exposed, legs longer, the femora generally reaching beyond sides of body, prothorax widest near the middle.

1. Prothorax margined at base..... 2
Prothorax immargined at base, or at most only slightly laterad..... 7
2. Abdominal coxal plates entirely obsolete..... 3
Abdominal coxal plates distinct and forming are reaching not more than to middle of segment; front basitarsi of male dilated..... 6
3. Mesosternal coxal plates obsolete..... 4
Mesosternal coxal plates distinct; claws simple.....*Naemia* Mulsant
4. Tarsal claws bifid or with a quadrate basal tooth..... 5
Tarsal claws simple; elytra vittate.....*Paranaemia* Casey
5. Claws with a quadrate basal tooth; prothorax rather strongly arcuate at base, with the raised marginal line complete.....*Colcomegilla* Timberlake
Claws bifid, the inner tooth much shorter and stouter than the outer tooth; prothorax subtruncate at base, with the raised marginal line distinct except at middle
Eriopsis Mulsant
6. Claws slender, with a quadrate basal tooth; third antennal joint of male longer than joint on either side, triangularly enlarged toward apex and ciliate on outer margin at apex*Ceratomegilla* Crotch
Claws bifid, the inner tooth nearly as acute as outer tooth but much shorter; third antennal joint of male simple and no longer than following joint.....*Adonia* Mulsant
7. Claws bifid or with a basal quadrate tooth..... 8
Claws simple 9
8. Claws bifid; mesosternal and abdominal plates obsolete or rudimentary
Hippodamia Chevrolat
Claws with a basal quadrate tooth; third antennal joint of male about equal to following joint and triangularly enlarged toward apex; front basitarsi of male swollen
Semiadalia Crotch
9. Body oval; side margins of elytra rather broadly reflexed, the epipleura horizontal; elytra maculate*Anisosticta* Chevrolat
Body extremely elongate; elytra vittate, the side margins narrowly reflexed and the epipleura sloping upward from within.....*Macronaemia* Casey

ANISOSTICTA CHEVROLAT

Anisosticta borealis new species

Differs from *A. bitriangularis* (Say) in having underside of body black, with anterior margin of prosternum narrowly white. Mesepimera black. Metepimera black, or at most very obscurely whitish. Venter entirely black. (In *bitriangularis* the mes- and metepimera and sides of venter are white.) Pronotum black, with narrow lateral and anterior border yellow. The anterior pale border widened at middle and uniting more or less with a median pale line reaching to the center. Basal margin with a semioval pale spot at middle. Elytra with a common black sutural vitta reaching almost to apex and dilated four times, *i.e.*, confluent with spots 3, 6, 8 and 9. Spots 1, 2, 4, 5 and 7 enlarged and confluent to form a broad sinuous vitta, which is sometimes narrowly joined to spot 9. Spot 7 sometimes free. Spot 3 sometimes joined to 1, and 6 to 4, thus isolating two pale areas.

1 male (holotype), Nulato, Alaska, June 14, 1916 (Harrington), and 2 paratypes, Anchorage, Alaska, June 6, 1917 (J. S. Hine), in U. S. National Museum.

NAEMIA MULSANT

Naemia seriata seriata (Melsheimer)

Specimens of this species in the U. S. National Museum are from "Mass.;" Staten Island, N. Y., Long Island, N. Y.; "N. J.," Cape May, N. J.; Chesapeake Beach, Piney Point, Breton Bay, etc., Md.; Fort Monroe, Old Point Comfort, Wachapreague and Ragged Point, near Coles Pt., Va.; Cameron, Riverside and New Orleans, La.; Victoria, Texas on cotton, and Brownsville, Texas. In the American Museum are specimens from Woods Hole, Mass.

Naemia seriata decepta (Blatchley)

In the American Museum from Beaufort, S. C., and the following places in Florida: Everglade, Kissimmee, Sanford and Dunedin. From Cuba: San Carlos Est., Guantanamo, Cubanäs; and from Sources Puantes, Haiti. In the U. S. National Museum from Biscayne, Capron, Pta. Gorda, Paradise Key, Fla. and St. Marc and Etang Sumetre, Haiti.

The Cuban and Haitian specimens, especially the latter, average somewhat larger than typical *seriata* and are brighter colored than Florida specimens of *decepta*, with which they agree in the reduced markings.

COLEOMEGILLA TIMBERLAKE

TABLE OF THE RACES AND VARIETIES OF *C. MACULATA* (DE GEER)

- | | |
|---|-------------------------|
| 1. Head weakly punctured (northern Mexico and northward)..... | 2 |
| Head distinctly and unequally punctured (Mexico, South America, and West Indies) | 4 |
| 2. Spots 2 and 3 united to form a large oval black mark, usually about equally distant from suture and side margin; spot 5 nearly always united with fellow on suture; spot 6 not touching suture even when enlarged; pronotal marks large, obtriangular, rarely confluent at their middle..... | 3 |
| Spot 2 usually free, forming a smutting detached black dot near side margin, or altogether absent, spot 3 oval, rather small and widely separated from spots 1 and ½; spots 4, 5 and 6 reduced, subequal, but sometimes one or another one-half as large as others, 5 free from suture; pronotal marks reduced, oval, or more or less curvilinear, and generally reaching about three-fourths from base. | |
| <i>fuscilabris</i> (Mulsant) | |
| 3. Median lobe of tegmen (male genitalia) slender, with a narrow truncate apex which is hardly notched..... | <i>lengi</i> Timberlake |
| Median lobe of tegmen somewhat broader, more fusiform, the apex a little wider and distinctly notched | <i>strenua</i> (Casey) |
| 4. Pronotal marks normally separated by a pale narrow median line or confluent only at the middle | 5 |
| Pronotal marks united, emarginate in front medially and broadly separated from anterior margin by a pale border and more narrowly separated from the side margins; elytral markings similar to <i>fuscilabris</i> , except that spot 2 is sometimes partially or even entirely separated from 3, and spots 4, 5 and 6 are subequal, moderately large, with both 5 and 6 usually united with their fellow on suture..... | <i>cubensis</i> (Casey) |
| 5. Elytral spot 6 free from suture, the markings similar to those of <i>fuscilabris</i> , except that mark 2 + 3 is more irregular, with separation of 2 more or less, rarely entirely, complete | 6 |
| Elytral spot 6 joined with its fellow on suture..... | 9 |
| 6. Legs black, size and markings similar to <i>lengi</i> , the pronotal marks sometimes reduced as in <i>fuscilabris</i> | 7 |
| Tibiae mainly reddish brown; pronotal marks bifurcate, the outer spur slender; spot | |

2 of elytra longitudinally lengthened and nearly separated from 3, which is large and quadrate; spot 5 narrowly separated from suture; size very large (8 mm.)

tibialis n. var. or subsp.

7. Spot 5 uniting with fellow on suture..... 8
Spot 5, as well as 6, free from suture, as in *fuscilabris*.....*bis-sex-punctata* (Latreille)
 8. Markings agreeing closely with *lengi* and *strenua*, but head distinctly punctured, male genitalia much like *strenua*.....*medialis* (Casey)
Agreeing with *medialis* but elytral markings much heavier, the black spots large, more or less narrowly separated one from the other.....*limensis* (Philippi & Philippi)
 9. Mark 2 + 3 reaching to side margin of elytra..... 10
Mark 2 + 3 free from side margins of elytra, and about as in *lengi*; spots 1, 4, 5 and 6 large, 5 and 6 being united with fellows on suture; pronotal marks nearly evenly obtriangular and narrowly separated from front margin.....*boliviensis* n. subsp.
 10. Similar to *boliviensis* except mark 2 + 3 reaches to side margin and pronotal marks a little larger, more narrowly separated from each other and sometimes confluent at the middle; venter entirely black, or obscurely pale at sides of segments 3 to 5
maculata (De Geer)
- Similar to *maculata* except that the suture is narrowly black between spots $\frac{1}{2}$ and 5 and more broadly black between 5 and 6; mark 3 triangular, with one angle directed backward between 4 and 5; 2 joined with 3 but not very broadly; pronotal marks obtriangular and bifid, both arms being short, blunt, subequal; venter entirely dark.....*maculata*, var. *triangulifera*, n. var.
- Similar to *maculata*, but elytral marks confluent; spot 1 nubilously joined to 2 + 3, latter continuous from suture to side margin and dilated at the middle behind to unite with 4; 4 narrowly joined to 5; suture continuously black from base to spot 6.....*maculata*, var. *confluens*, n. var.

***Coleomegilla maculata fuscilabris* Mulsant**

Megilla maculata floridana Leng.

In the American Museum represented by specimens from Key West, Lakeland, Sanford, Royal Palm Park, and La Belle, Florida. In the U. S. National Museum from Paradise Key, Coral Gables, Gainesville, Moore Haven, Enterprise, Orlando, Capron and Lake Poinsett, Florida, and New Orleans, Covington and Mandeville, Louisiana. The type locality is New Orleans.

A series in the National Museum from Shaw Pond and Eastern Branch, Washington, D. C. (H. S. Barber) shows intergradation with *lengi*, but ranges closer to *fuscilabris*. The markings are larger than in typical *fuscilabris*, with spot 5 sometimes joined with fellow on suture. Spot 2 present and usually confluent with 3. In typical *fuscilabris* spots 5 and 6 are always small, generally smaller than 4 and either one or both may be absent. Washington, D. C., is further north than we would expect the influence of *fuscilabris* to show, and Mr. Barber suggested to me that possibly specimens had been introduced on water plants from Florida.

***Coleomegilla maculata lengi* Timberlake**

This race is generally distributed east of the Rocky Mountains. The type locality is Columbus, Ohio. Specimens of *Coleomegilla* from Arizona and southern California (Imperial Valley and San Diego) are presumably referable to *strenua*.

***Coleomegilla maculata strenua* (Casey)**

In the National Museum are specimens from Camargo, El Molina, Los Mochis, Rosario, Eldorado, Tuxpan and San Jose de Guaymas, Mexico. In the American Museum is a specimen from Paso del Norte, Chihuahua. The type locality is Brownsville, Texas.

The specimen from Paso del Norte has markings as in *strenua*, but head is strongly punctured. Two specimens from Rosario, Sinaloa, have the markings nearly as in *strenua* and the head rather strongly punctured. Three specimens from Tuxpan have marking more as in *medialis* and head rather strongly punctured. The Tuxpan specimens are perhaps better referred to *medialis*.

***Coleomegilla maculata medialis* (Casey)**

This seems to be the *Coccinella oblonga* Olivier, 1791, but that name is pre-occupied.

In the American Museum are specimens from Jalapa and Mazatlan, Mexico. In the National Museum it is represented by specimens from San Juan Bautista, Tabasco; Oaxaca; St. Lucrecia, Vera Cruz; and Colima, Mexico; Dueñas and Paso Antonio, Guatemala; Honduras (type, in Casey collection); Gatun, La Chorrera, Tabernilla, Lion Hill, Ancon, Summit (Canal Zone), Chagres River, Juan Mina, Chilibre River, one mile above Juan Mina, etc., Panama; Medellin, Colombia; Caracas and San Carlos Cojedes, Venezuela; Coroni River, Trinidad; and Martinique.

In one out of six specimens from San Carlos Cojedes (H. P. Pittier) spot 2 reaches the side margin, thus agreeing with *maculata* (De Geer), but the pronotal black mark is very large, entire, leaving only the side margins and outer portion of anterior margin on each side, narrowly pale. The others agree closely with *medialis*. In one out of three specimens from Coroni River, Trinidad (Harold Morrison), spot 2 reaches rather obscurely to the side margins.

***Coleomegilla maculata bis-sex-punctata* Latreille**

As recognized by me this agrees with *medialis* except that spot 5 is reduced in size and separated from suture. It is questionable whether the specimens seen by me represent a race or a mere variation of *medialis*.

It is represented by two specimens from Panajachel, 5,000 ft., Guatemala (Champion), in the American Museum.

***Coleomegilla maculata tibialis*. new subspecies**

Differs from other races in having tibiae distinctly brown, the hind pair only distinctly so on the outer (dorsal) margin. Head strongly punctured. Pronotal marks strongly obtriangular and bifid in front, with outer spur slender, the inner one shorter and truncate. The two marks well separated medially, broadly behind and one-half closer on anterior half. They are broadly separated from anterior and lateral margins. Elytral marks 1 and $\frac{1}{2}$ large but well separated from each other and from 2 + 3. Mark 2 + 3 almost divided by a thin obscure pale line, the component 2 being elongate and longitudinally disposed. 3 large and quadrate, forming a deep acute angle in the pale ground color where it joins with 2 behind, and acutely produced along 2 on its other side. 2 free from the side margin. Spot 4 rather large and round. 5 smaller than 4 and reaching close to but not quite touching suture. Spot 6 about one-half as large as 5 and free from suture. Length, about 8 mm.

Described from 1 male (holotype), Rio Frio, Colombia, Mar. 1924 (H. W. Atkinson), in U. S. National Museum.

***Coleomegilla maculata limensis* (Philippi & Philippi)**

This as recognized by me agrees closely with *medialis* but has the elytral markings much heavier.

Pronotal marks obtriangular, slightly emarginate at apex, well separated medially, and reaching to apical fifth. Elytral marks all large, but 2 not reaching side

margins and 6 not confluent with fellow at suture. Mark 2 + 3 emarginate on each side where the two components are fused, 3 being much the larger, extending angularly forward between spots 1 and $\frac{1}{2}$ and behind approaching in a broad curve to spot 4 or even slightly confluent with 4. Head strongly punctured.

5 specimens from Peru, without further data, in the American Museum.

3 specimens from Milagro, Ecuador, Nov. 29, 1922 (F. X. Williams), in H.S.P.A. collection, agree closely with the Peruvian specimens. In two of these mark 2 + 3 is irregularly crescent-shaped, with inner horn very much longer than the outer part. In the other specimen mark 2 + 3 is truncate in front, spot 1 prolonged behind and nebulously confluent with 2 + 3, which is distinctly confluent with 5.

***Coleomegilla maculata boliviensis* new subspecies, or var.**

This as described in the table does not differ much from *maculata* (De Geer) except that mark 2 + 3 is comparatively small and fails to reach the side margin.

Described from 1 specimen (holotype) from Rurrenabaque, Rio Beni, Bolivia, Jan. (W. M. Mann), in U. S. National Museum.

***Coleomegilla maculata maculata* (De Geer)**

Pronotal marks large, narrowly separated medially, sometimes a little coalescent at middle, and narrowly separated from anterior and lateral margins. Elytral markings heavy, but normally showing little if any coalescence. Mark 2 + 3 completely fused and extending to the side margins. Spot 6 fused with fellow on suture. Head strongly punctured.

In U. S. National Museum represented by specimens from Pernambuco, Jan. and Feb. 1883 (Koebele), Manaos (Miss H. B. Merrill) and Obidos (H. Rolle), Brazil; Paramaribo, Dutch Guiana (D. F. Fernandez); Ciudad Bolivar, Venezuela, 100 ft., Nov.-Dec. 1929 (Ernest Holt); and Barbados. In American Museum, 5 from Brazil without further data, 2 from Obidos (H. Rolle) and 13 from Barbados.

The Barbados specimens show a strong tendency toward coalescence of the pronotal marks, which are sometimes completely joined as in *cubensis* (Casey), but they are much larger than in that race and reach nearly to anterior margin. In one specimen mark 2 + 3 does not quite reach to the side margin. The variation of the Barbados specimens is in the direction of *cubensis*, but they are clearly referable to *maculata*.

The Obidos specimens are unusually large (about 7.25 to 7.8 mm.) and have the pronotal marks smaller than usual, obtriangular, slightly emarginate anteriorly and well separated medially. Elytral marks normal for *maculata*.

***Coleomegilla maculata maculata* var. *confluens* new variety**

One out of six specimens from Obidos is smaller than the others and has the black markings of elytra confluent.

Pronotal marks about the same as in the other Obidos specimens described above. Suture of elytra broadly margined with black from base to spot 6 so that the usual four sutural marks are distinctly joined. Spot 1 nubilously connected with 2 + 3. Mark 2 + 3 continuous and nearly even from side margin to suture, except at the middle behind it is triangularly dilated and extends back to join broadly with 4. Spot 4 narrowly confluent with 5. Spot 6 forming with fellow a transverse band that crosses the suture and reaches almost to side margins. By this coalescence a pale mark shaped like half an hour-glass is isolated at the base, and behind the middle an oblique pale streak is fully enclosed.

Described from one specimen (holotype) from Obidos, Amazonas, Brazil (H. Rolle), in Casey collection, U. S. National Museum.

***Coleomegilla maculata maculata* var. *triangulifera* new variety**

Differs from typical *maculata* in its rather smaller size, smaller pronotal marks and peculiar shape of the elytral marks.

Prothorax pale yellowish or whitish, distinctly paler than the flesh colored elytra. Pronotal marks obtriangular, distinctly emarginate in front, well separated from anterior and lateral margins, and broadly separated from each other behind but coming close together in front. Mark $\frac{1}{2}$ of elytra large and lozenge-shaped, the suture behind it to mark 5 + 5 being obscurely blackened. Spot 1 large but well separated from the others. Of mark 2 + 3 the component 2 is relatively small and transversely lengthened to side margin. Component 3 large, triangular, transverse in front and angularly extended behind between 4 and 5. Spot 4 large and narrowly separated from 5. Spot 5 large and broadly confluent at suture with fellow. Spot 6 transverse and reaching to suture where it joins with fellow. Marks 5 + 5 and 6 + 6 distinctly joined by a black sutural line. Length, about 5 mm.

Described from 2 specimens (holotype and paratype) Ciudad Bolivar, Venezuela, 100 ft., Nov.-Dec. 1929 (Ernest Holt), in U. S. National Museum.

Another specimen with the same data is a typical *maculata*.

***Coleomegilla maculata cubensis* (Casey)**

Pronotal marks consolidated, broadly separated from anterior and lateral margins and strongly emarginate in front medially. Elytral marks medium in size, 1, 2 + 3, 4 and 6 well separated from each other. Component 2 sometimes separated from 3, or partially separated, or the two fused to form an oval mark, never reaching to side margin. Spot 5 and 6 fused more or less at suture with fellow. Mark 5 + 5, 6 + 6 and 4 about equal in size. Head strongly punctured.

Represented in National Museum by specimens from Cayamas, Santiago de las Vegas, Jobabo, Manati and Baragua, Cuba; Haiti and Camp Perrin, Haiti; and near San Pedro de Macoris, Dominican Republic. In American Museum from near Havana, 24 K. north of Viñales and 12.5 K. south of Pinar del Rio, Cuba; and La Morimière, Haiti.

The related *Colcomegilla innotata* (Mulsant) is known from Puerto Rico. This is hardly more than an insular race of *maculata*.

HIPPODAMIA CHEVROLAT

TABLE OF AMERICAN SPECIES OF THE *13-PUNCTATA* GROUP, BASED ON MALE GENITALIA

1. Median lobe of tegmen with a rod-like, well-chitinized sublaminar process on each side from lateral margin of ventral wall. 2
 Median lobe of tegmen shorter, slightly tapering from base to beginning of the last third, where it is abruptly bent downward and rapidly tapers to acute apex, process from ventral wall on each side membranous, broad, thinly laminate and with a short acute apical point, ventral plates of the lobe ending apically in recurved rather slender points which cross each other and project on each side close to apex of lobe; paramera rather short, broad, ciliate on dorsal margin to the middle; siphon bent almost double near end of basal third, the part beyond abruptly and considerably thickened, becoming more compressed and dilated into a large triangular lobe on upper margin near beginning of apical third, the part beyond the dilation slender
amerioana Crotch
2. Process on each side of median lobe of tegmen long and slender, ending opposite the

1 ♂, White Fish Point, Lake Superior (Hubbard & Schwarz)

beginning of the last fifth of the lobe, and slightly hooked on outer side at apex; basal third of dorsal surface of median lobe with a triangular crest, with almost vertical side walls; lobe bent downward just beyond apex of triangular crest at angle of about 45°, slightly expanding in its apical fourth with the dorsal surface concave; apex of the lobe rounded, with a small blunt median point; paramera rather long and broad, but narrowed toward base, the apex with a thin membranous margin; siphon abruptly bent near end of basal third, the part beyond slightly and fusiformly swollen, armed with a very small membranous lobe near beginning of the last eighth, the apical eighth slender.....*falcigera* Crotch

1 ♂, Hudson Bay (Hubbard & Schwarz collection)

Process on each side of median lobe vertically laminate, finger-like, ending opposite beginning of apical third of the lobe; dorsal side of lobe with a very small triangular raised area at base, subdepressed beyond and abruptly bent downward at an angle of 45° a little beyond the middle; paramera broad and moderately long, narrowed only at extreme base; siphon bent almost double near end of second fifth of the length, much as in *falcigera* but less swollen in middle portion, and with the dorsal dilation near apex larger.....*tibialis* (Say)

COCCINELLINA TIMBERLAKE

TABLE OF NEOTROPICAL SPECIES FORMERLY INCLUDED IN *COCCINELLA*

- | | |
|---|-----------------------------|
| 1. Elytra immaculate flavous or reddish..... | 2 |
| Elytra marked with black..... | 4 |
| 2. Disk of pronotum black, with a white spot on each side..... | 3 |
| Disk of pronotum black, broadly bordered in front and on sides with yellowish white; anterior margin of black area of pronotum transverse in female, indented on each side in male; elytra red or tawny; mesepimera white; legs black; length, 5.2 mm.
<i>fulvipennis</i> (Mulsant) | |
| 3. Pronotum bordered on sides and in front with white, the border narrow and nearly uniform in width throughout; elytra reddish yellow or flavous; mes- and metepimera white; length, 4 mm..... | <i>emarginata</i> (Mulsant) |
| White border of pronotum extremely narrow in front, distinctly notching the black medially, and much broader on the sides but constricted just behind the middle by a blunt extension of the black area; mesepimera white; length, 3.3 to 4.8 mm.
<i>ecuadorica</i> n. sp. | |
| 4. Pronotum with a pair of discal spots..... | 5 |
| Disk of pronotum black, without discal spots..... | 7 |
| 5. Black marks of elytra not confluent with sutural vitta..... | 6 |
| Black marks of elytra confluent with sutural vitta and dividing the surface into five pale areas: two basal, two median, and one apical; discal spots of pronotum elongate; pronotum margined with yellow in front and more broadly on sides; length, 4.5 mm. | <i>petitii</i> (Mulsant) |
| 6. Black sutural vitta dilated at the beginning of the first third and again angularly near apex; elytra each with two black discal marks, one on the callus, the other at two-thirds; length, 3.9 mm..... | <i>lucasi</i> (Mulsant) |
| Black sutural vitta angularly dilated a little behind the scutellum and near the apex, and curvilinearly contracted at the middle; elytra each with two large black marks, longitudinally disposed on disk and often confluent; length, 3.9 mm.
<i>ancoralis</i> (Germar) | |
| 7. Elytra red or yellowish red, with three rounded yellowish white spots enclosed in a black circle; pronotum broadly yellowish white on each side, but the pale border constricted before the middle by a lateral extension of the black area; length, 4.5 mm.
<i>pulchella</i> (Mulsant) | |
| Elytra not thus marked..... | 8 |
| 8. Each elytron pale around the periphery..... | 9 |

Elytra with sutural margin and two transverse bands, black; first band broad, curving forward to callus, with a lateral spur directed backward, the hind margin of the band slightly behind the middle; second band strongly oblique and subapical, also with a backward-directed spur on its outer margin; pronotum black, with anterior margin narrowly pale, and lateral margins a little more broadly so; length, 4 mm.

shannoni n. sp.

9. Elytra each with two black marks or abbreviated bands, more or less distinctly contracted toward outer and sometimes confluent on inner side.....*eryngii* (Mulsant)

Elytra black, but flavous around the entire periphery of each and marked with four somewhat rounded spots, one next to the scutellum, two a little before the middle and one subapical.....*arcata* (Mulsant)

Coccinellina emarginata (Mulsant)

In the U. S. National Museum this species is represented by specimens from Mexico as follows: Dist. Fed. (J. R. Inda, R. Miller, J. Conradt); Mexico City (O. W. Barrett); Digna (A. L. Herrera); Jalapa (J. T. Mason); Tlalpam (R. H. Hay); Aguas Calientes (E. A. Schwarz); Sierra de la Ajusco (R. H. Hay); Tacuba, on beans (H. F. Wickham); Cuernavaca (E. G. Smythe); Cordoba (F. Knab); Amula, Guerrero, 6,000 ft. (H. H. Smith). From Nicaragua: Managua (A. D. Harvey); San Marcos (Baker). From Costa Rica: San José (M. Valeris); Zarzero (Schild & Burgdorf); Tilaran, Guanacaste, 550 M. alt. (P. C. Standley). Also from Tegucigalpa, Honduras (F. J. Dyer); Merida, Venezuela (S. Bricano); and Campa Santos de Salto, Argentina, in citrus grove (M. Kisliuk). In collection of the H.S.P.A. Experiment Station are specimens collected by F. X. Williams at Mera and Baños, Ecuador.

The Argentine specimen is a little larger than usual, but not separable on the basis of a single specimen.

Coccinellina ecuadorica new species (Plate II, Fig. 31)

Similar to *C. emarginata* (Mulsant) but pronotum unequally bordered in front and on sides with white, and only the mesepimera, white. The male genitalia are distinctive. Median lobe of tegmen subdepressed, slightly convex above, about two and one-half times longer than wide, widest close to the base, then tapering slightly to beginning of apical third, beyond which it is a little expanded and strongly curved upward. Apex obliquely truncate on each side, so that the margins meet in an angle of 90°, but angle a little rounded off. On dorsal surface on each side before the upturned part is a long line of hairs. Paramera straight, nearly as long as median lobe, moderately broad, rounded at apex and ciliate on each margin. Sipro short, rather stout, and abruptly contracted close to apex into a short slender portion. In *C. emarginata* the median lobe of tegmen is not so broad, regularly tapering from base to apex to form the outline of a very acute-angled triangle. Apical third upturned. Dorsal surface of basal part strongly tectiform, but the ridge narrowly creased in median line. At apex of tectiform part is a pencil, or short line, of hairs submedianly on each side. Paramera and sipro considerably slenderer than in *ecuadorica*, but otherwise not much different.

1 male, 2 females (holotype ♂, allotype and paratype), Huigra, Ecuador, 4000 ft. (F. X. Williams), in collection of Hawaiian Sugar Planters' Experiment Station; and 2 males (paratypes), Cariamanga, Ecuador, on cotton (C. H. T. Townsend), in U. S. National Museum.

Coccinellina shannoni new species

Suggestive of *C. petiti* (Mulsant), and possibly only a variety of that species, but lack of discal spots on pronotum is probably distinctive. Other characters as given in table.

1 female (holotype), Matucana, Peru (R. C. Shannon), in U. S. National Museum.

PSEUDADONIA NEW GENUS

In general form and shape of the head, prothorax and elytra this agrees closely with *Coccinella*, having a broader form than in *Semiadalia*. In some respects it agrees with *Semiadalia*, as in the immargined base of pronotum, dilated tarsi and structure of claws, but differs in having the abdominal plates incomplete, the third antennal joint simple, pronotum more convex and wider behind the middle, mesosternum much more depressed, etc.

Claws moderately long, with a basal quadrate tooth. Legs hardly elongate, the tarsi distinctly shorter than tibiae. First two joints of front and middle tarsi dilated in male, but rather less broadly than in other genera showing this character. Antennae with joints 3 to 8 slender, cylindrical, the third about four times longer than thick and not dilated at apex as in *Semiadalia* and *Ceratomegilla*. Club rather elongate, enlarging from the base of ninth joint to the obliquely truncate apex of the eleventh. Sternal and abdominal plates well developed, the latter incomplete, with the line curving outward very close to the hind margin of the segment and without oblique line. Mesosternum depressed, truncate in front with a raised margin. Middle coxae rather narrowly separated, the metasternal process narrowly rounded between them. Prothorax rather strongly convex, widest behind the middle, arcuate behind and not at all margined at base. Head well exposed. Form rather broadly oval, the elytra each hardly more than twice longer than wide. Epipleura of elytra horizontal and of ordinary width.

Genotype: *Pseudadonia chiliana* n. sp.

***Pseudadonia chiliana* new species**

Male—Head, under surface of body and base of pronotum black. Face with a broad transverse white band between the eyes. Mesepimera and epipleura of prothorax white. Pronotum broadly white in front and at sides, the pale color reaching to middle in median line. Black basal area of pronotum strongly trilobed at apex, the two lateral lobes moderately wide, somewhat tapering and directed slightly obliquely outward as well as forward, the median lobe much broader, rounded at apex but with a rather deep notch in the middle. At apex of the lateral lobes on each side there is an extension of the black color straight outward, which reaches half way across the white border (and which may possibly form a detached spot in some specimens). Scutellum black. Elytra fulvous, unmarked. Labrum black. Slender intermediate joints of antennae pallid, but the base and club blackish. Outer margin of mandibles, whitish. Length, about 4.9 mm., width, about 3.8 mm.

The genitalia show no particular close alliance to *Coccinella* or any of the genera with enlarged tarsal joints. Paramera broad, only a little shorter than median lobe of tegmen, about twice as long as wide, with outer margin straight, inner margin arcuate and ciliate from apex to the middle. Median lobe depressed, rather broad, about three times as long as wide and ogivally rounded at apex. Siphon moderately long, abruptly but not strongly bent at the beginning of the last third, which is cylindrical, moderately tenuous and hardly tapering except very close to apex.

Described from 1 male (holotype) from southern Chile (M. J. Rivera), in U. S. National Museum.

TABLE OF AMERICAN GENERA OF COCCINELLINAE WITH METACOXAL PLATE INCOMPLETE

1. Epipleura of elytra horizontal and never much expanded.....	2
Epipleura more or less inclined and descending externally or very broad.....	6
2. Mesosternum depressed and truncate in front.....	3
Mesosternum either convex or emarginate medially in front.....	5
3. Metacoxal plate without an oblique line.....	4
Metacoxal plate divided by an oblique line, which is very distinct and meets the bounding line very close to hind margin of segment; antennae short, the apical joint as long as wide; elytral punctures generally very fine and equal; form oval, or moderately elongate oval, and convex; pronotum always black with anterior corners and sometimes anterior border, pale.....	<i>Coccinella</i> Linnaeus

4. Front and middle tarsi or male simple; form oval, moderately convex, elytral punctures very fine and equal; mesosternum sometimes slightly sinuate medially in front; pronotum black, with a narrow pale border in front and on sides and sometimes with two discal spots.....*Coccinellina* Timberlake
- Front and middle tarsi of male dilated; form rather broadly oval, convex; mesosternum truncate in front, with a raised margin; black area of pronotum strongly trilobed*Pseudadonia* Timberlake
5. Mesosternum strongly emarginate medially; prosternal process broad and bicarinate, the carinae reaching forward to middle of segment; epipleura about as wide as space between middle coxae, which are rather broadly separated; metacoxal plate without an oblique line, antennae elongate, the apical joint much longer than wide, squarely truncate at apex; elytra with fine and strong punctures interspersed; form broadly oval and convex.....*Anisocalvia* Crotch
- Mesosternum convex and slightly sinuate medially in front; prosternal process slender and not carinate; epipleura more or less evidently furrowed, distinctly wider than space between middle coxae, which are close together; antennae elongate, the apical joint about as long as wide, longer than penultimate joint and slightly obliquely truncate at apex; elytral punctures more or less coarse, or if finer on the disk, having those on outer margin coarser; form more or less broadly oval and subdepressed.....*Mulsantina* Weise (*Pseudocleis* Casey)
6. Epipleura of elytra very wide, obviously exceeding width of space between outer margin of middle coxal cavity to outer margin of sternum; elytra with a broad explanate margin 7
- Epipleura moderately wide, not or hardly exceeding width of space from middle coxal cavity to outer margin of sternum..... 8
7. Elytra immargined on outer border; epipleura equalling or exceeding one-half the width of whole sternum and strongly descending externally, mesosternum somewhat emarginate in front medially; intercoxal process of first ventrite subtruncate at apex; oblique line of metacoxal plate subobsolete; large and orbicular, the elytra with a very wide explanate margin.....*Mononeda* Crotch
- Outer border of elytra with a more or less evident raised margin; epipleura and explanate margin of elytra often not so greatly widened as in *Mononeda*; intercoxal process of first ventrite rounded at apex; oblique line of metacoxal plate either distinct or nearly obsolete; otherwise much like *Mononeda*.....*Neda* Mulsant
8. Scutellum depressed, level with surface of elytra..... 9
- Scutellum distinctly elevated above surface of elytra and declivous in front; antennae abruptly widened at the ninth joint, the first two joints of club produced into a tooth on inner side, the apical joint about as long as wide and produced into a broad truncate joint on inner margin; mesosternum rather convex and emarginate medially in front; prosternal process expanding behind the coxal cavities; epipleura of elytra widest at end of first third, then gradually tapering to an acute point behind; callus of elytra very prominent.....*Pelina* Mulsant
9. Mesosternum convex; elytral punctures rather strong and close..... 10
- Mesosternum at most weakly convex between and just in front of middle coxae..... 11
10. Mesosternum with a small obtuse emargination in front medially; prosternal process carinate on each side; oblique line of metacoxal plate meeting the bounding line very obliquely, so that the outer angle is very acute; elytral punctures moderately strong and unequal; last two antennal joints wider than long, the apical one truncate at apex.....*Neoharmonia* Crotch
- Mesosternum truncate in front, or with a mere trace of an emargination medially; metacoxal plate without an oblique line; apical joint of antenna quadrate, as long as wide, truncate at apex.....*Harmoniaspis* Casey
11. Prothoracic epipleura not foveate; antennae with club well developed..... 12
- Prothoracic epipleura with a rounded depression on inner side anteriorly; antennae short, slender, gradually and only slightly increasing in thickness beyond the middle; the apical joint longer than wide and rounded at apex; mesosternum with a deep triangular emargination in front; metacoxal plate attaining hind margin

of segment; epipleura of elytra descending externally and slightly foveate to receive hind femora.....*Coelophora* Mulsant*

12. Frons rather more than twice as wide as diameter of eye; eyes finely faceted, the inner orbits parallel 13
 Frons less than twice as wide as diameter of eye; eyes more coarsely faceted, with inner orbits more or less convergent behind..... 14
13. Mesosternum truncate in front, or at most slightly sinuate in middle; oblique line of metacoxal plate obsolete; form rounded, very convex; pronotum black, the anterior and lateral margins and two discal spots (often confluent with pale margin), white; elytra immaculate*Cycloneda* Crotch
 Mesosternum rather strongly but obtusely emarginate; oblique line of metacoxal plate frequently distinct but incomplete; elytra finely or moderately strongly punctured, form broadly oval to orbicular, rather less convex than in *Cycloneda*; pronotum pale with seven black spots forming an M (the mediobasal spot sometimes absent), or in melanic form black with sides broadly pale; elytra maculated, or in melanic form black with a pale mark before the middle.....*Olla* Casey
14. Frons distinctly exceeding diameter of eye, but less than twice as wide..... 15
 Frons no wider than diameter of eye, eyes a little more coarsely faceted than in *Paraneda*; antennae moderately long; claws small, hardly longer than basal quadrate tooth; prosternal carinae reaching nearly to anterior margin of segment; mesosternum less distinctly emarginate in front medially than in *Paraneda*

Erythroneda new genus

Type, *Daulis rubida* Mulsant

15. Elytra coarsely punctured, dark brassy green; pronotum red becoming somewhat paler on each side; claws short, no longer than basal tooth; frons nearly twice as wide as diameter of eye; mesosternum with a small obtuse emargination in front and a small fovea on the declivous front edge; oblique line of metacoxal plate incomplete

Chloroneda new genus

Type, *Cycloneda metallica* Crotch

Elytral punctures very fine; claws large, much longer than basal quadrate tooth; mesosternum slightly emarginate in front and foveate on the declivous edge; frons posteriorly about one and one-half times wider than diameter of eye; elytra immaculate; pronotum pale at sides, darker in middle, the dividing line between the two areas strongly arcuate and black.....*Paraneda* Timberlake

Type, *P. viridescens* Timberlake

KEY TO COELOPHORINE GENERA, BASED ON MALE GENITALIA

1. Siph simple, or at most a little swollen between the middle and beginning of the last third 2
 Siph strongly swollen at or beyond the middle and armed with a pair of retrorse hooks or triangular membranous processes, originating near apex of the swelling from the ventral wall; or sometimes only slightly swollen but having the lateral margins erenulated before the ventral processes..... 11
2. Siph of ordinary length..... 3
 Siph extremely long and slender, somewhat tapering in apical part, the apex being moderately tenuous; median lobe of tegmen compressed, strongly so at its upturned apex; its dorsal surface narrow, with a low median crest on the basal fifth, its apex in side view subtruncate and somewhat resembling the prow of a canoe; paramera almost contiguous at base, moderately armed, somewhat clavately enlarged at apex.....*Heterocaria* new genus
- Type, *H. papuana* n. sp. (Laloki, Papua, F. Muir)
3. Median lobe of tegmen more or less emarginate at apex..... 4
 Median lobe of tegmen acute or truncate at apex, never emarginate..... 5

* *Coelophora* is not American, but in the U. S. National Museum is a specimen from Central America, and the record is apparently authentic. The species as I remember, is *C. inaequalis* var. *9-maculata* (Fab.). It must have been introduced through commerce.

4. Median lobe depressed, only slightly longer than wide, deeply emarginate at apex, and acutely angled on each side of the emargination (form resembling an old-fashioned bootjack and recalling that of *Synharmonia*, but the lobe much shorter, not turned upward at apex and much more deeply emarginate); paramera short and straight, as long as median lobe and well separated at base; siphon short, moderately stout, and having the apical tenth rather abruptly tapering to a fine membranous point
Type, *Coelophora guttata* Blackburn *Gyrocaria* Timberlake
Median lobe about three times as long as wide, with a small notch at apex and the apex conspicuously turned upward; paramera similar being short and straight, but much more evenly rounded at apex; siphon similar, but abruptly narrowed, its apical fifth being very slender and tapering to an exceedingly tenuous point. *Ocnopia* Mulsant
Type, *O. cinctella* Mulsant
5. Siphon stout, with the two dorsal strands conspicuously twisted one over the other near apex 6
Siphon slender, at least toward apex, so that the twist in the dorsal strands, although present, is not at all conspicuous. 8
6. Siphon without a constriction, the extreme apex more or less membranous (sometimes expanding) between the two pairs of chitinized rods. 7
Siphon constricted at the twist in the dorsal strands, the part beyond being rather long, abruptly bent downward and then abruptly recurved in a sharp S-shaped fashion; portion of siphon basad of the twist broad and depressed nearly to the base; median lobe of tegmen depressed, nearly four times as long as wide, and arcuately tapering to the acute, somewhat upturned apex; paramera as long as median lobe, arched toward base and inserted moderately far apart. *Synia* Mulsant
Type, *S. melanaria* Mulsant
7. Median lobe of tegmen elongate, but not narrow (a little more than four times as long as wide), tapering in the apical fourth, and with an abruptly and strongly upturned point, which is truncated at apex; ventral walls of median lobe meeting in the median line, but leaving a large oval opening for passage of siphon just before the upturned apex; paramera rather stout, as long as median lobe, almost straight and ciliate on both margins nearly to the middle. *Caria* Mulsant
Type, *C. dilatata* (Fabricius)
Median lobe narrow, elongate (about five times as long as wide), tapering to an acute point at apex which is just perceptibly and very briefly upturned; dorsal surface of median lobe with a carinate median crest on middle third of length and somewhat furrowed on each side of the crest, the two furrows uniting at basal end of crest and proceeding to the base; under surface of median lobe crested on apical third, the siphon issuing basad of this crest; paramera much as in *Caria*, but slightly arched and ciliate on inner margin only at apex and on outer side nearly to the base. *Cyphocaria* Crotch
Type, *C. duvaucelii* (Mulsant)
8. Ventral wall of siphon not swollen between the middle and beginning of the apical third; median lobe of tegmen depressed. 9
Ventral wall of siphon having the membranous part bulged out between the middle and beginning of the apical third; median lobe of tegmen rather narrow and elongate, tapering triangularly to acute apex and subcompressed (deeper at base than the dorsal width); paramera as long as median lobe, nearly straight, rather slender, and ciliate at apex and on outer side to the middle or a little beyond. *Lemnia* Mulsant
Type, *Lemnia saucia* Mulsant
9. Median lobe of tegmen very acute at apex; siphon moderately long and slender. 10
Median lobe of tegmen about three times as long as wide, parallel-sided, but narrowed near apex, the latter strongly curved upward and truncate; paramera rather stout, nearly straight, not quite as long as median lobe, little thickened at apex and ciliate on apical half and on inner margin nearly to the base; siphon short, moderately slender, abruptly narrowed near apex, and with the extreme apex membranous, slightly expanded and abruptly bent downward. *Protocaria* Timberlake
Type, *P. scalaris* Timberlake

10. Median lobe of tegmen slender, acicular; paramera slender, arched, fully as long as median lobe and cirrate on both sides of the apical third; siphon moderately long, slender and tapering to a tenuous point at apex.....*Propylea* Mulsant
Type, *P. 14-punctata* (Linnaeus)
- Median lobe of tegmen somewhat more than three times as long as wide, the sides subparallel in basal half, then ogivally narrowed, with a short, slender, upturned process at apex; paramera as long as median lobe, rather slender, strongly arched and cirrate at apex and on apical third of outer margin; siphon much as in *Propylea* but not so tenuous at apex.....*Spilocaria* new genus
Type, *Coclophora bissellata* Mulsant
11. Lateral margins of siphon not crenulated before the hooks or membranous processes. . 12
Lateral margins of siphon crenulated before the processes by means of one or two pairs of broadly rounded protuberances; processes of siphon membranous, appearing slightly retrorse as seen from above; portion of siphon beyond processes rather elongate and slender, abruptly bent downward at a slight constriction near its middle, the part beyond recurved and tapering to a fine point; the dorsal strands of apical portion of siphon twisted one over the other; median lobe of tegmen large, depressed, four or five times longer than wide, more or less ogivally pointed at apex; under surface of median lobe more or less carinate in median line on apical third, the carina ending basad at a more or less pronounced conical process; paramera slender, more or less curved, reaching to apex of median lobe, and cirrate on outer margin as far as or beyond the middle.....*Coclophora* Mulsant
Type, *C. inaequalis* (Fabricius)
12. Median lobe of tegmen depressed, with a broad dorsal surface..... 13
Median lobe of tegmen compressed, deep dorso-ventrally at base where it appears constricted in dorsal view, then either gradually tapering toward apex, as seen from side, or abruptly becoming depressed on apical half, with the dorsal surface ovally expanded in that portion; paramera straight, slender, not enlarged at apex; siphon rather short and stout, cylindrical moderately swollen near beginning of apical third, with the dorsal strands separating at the swelling, and armed beneath with a pair of membranous or more or less chitinated processes; portion of siphon beyond the swelling tapering, slightly constricted close to apex, with the part beyond bent slightly downward, more membranous, but not at all tenuous; dorsal strands of apical portion of siphon inconspicuously twisted.....*Eocaria* Timberlake
Type, *E. muiri* Timberlake
13. Median lobe of tegmen large, about three times, or more longer than wide, compressed beneath; siphon with comparatively weak or membranous processes from ventral walls at apex of swelling..... 14
Median lobe of tegmen short and broad, not much longer than wide, strongly depressed above and beneath, and ogivally narrowed to a very short projecting point at apex; paramera longer than median lobe, moderately slender, slightly curved and well separated at their bases; siphon rather short, moderately slender, cylindrical, more firmly chitinated than usual, with the separation of the component strands little apparent and with no evident swelling, but provided with a pair of processes near the beginning of the apical third, which are more or less chitinated and sometimes form large and conspicuous retrorse hooks.....*Phrynocaria* Timberlake
Type, *Coccinella congener* Schönherr
14. Median lobe of tegmen having a dull, microscopically tessellate, softer area at base of dorsal surface; under surface of lobe with a strong medio-longitudinal crest formed out of two appressed lamina, which separate for passage of siphon, but fuse in apical fourth and as seen from side become obliquely truncate to apex of lobe; paramera approximated at base, strongly curved, somewhat expanded toward apex and having their broader side parallel to surface of median lobe; siphon more or less swollen, with the two dorsal strands distinctly indicated, and having a pair of membranous processes from the ventral strands at the apex of the swelling; portion of siphon beyond ventral processes slender and having an inconspicuous twist in the dorsal strands.....*Bothrocalvia* Crotch

Type, *B. albolineata* (Schönherr)

Median lobe of tegmen entirely firm and polished above; siphon usually very strongly swollen; paramera more or less approximated at base, but soon spreading apart, their curvature more in a lateral plane instead of in a dorso-ventral direction as in *Bothrocalvia*, so that the flattened inner surface at apex of each is obliquely inclined to dorsal surface of median lobe; the parts otherwise much as in *Bothrocalvia* *Microcaria* Crotch

Type, *M. mulsanti* (Montrouzier)

HETEROCARIA NEW GENUS

Characters in general those of *Coelophora* Mulsant. Head as in *Coelophora* except that the frons is narrower and not greatly wider than width of eye. Pronotum indistinctly margined on each side. Disk of pronotum shallowly depressed or subfoveate on each side, close to lateral margin. Fovea of thoracic epipleura shallow, but reaching to the middle. Prosternal carinae reaching nearly to anterior margin, somewhat divergent behind, and the apex of the intercoxal process broad and rounded. Mesosternum weakly emarginate in middle of anterior margin, but the declivous anterior surface with a distinct pit to receive the prosternal process. Elytra as in *Coelophora* except that the lateral margins are definitely explanate, the disk somewhat furrowed just within the lateral margins, and the epipleura less strongly descending externally. Elytra with a distinct marginal bead on outer margin from base for about two-thirds of the length. Characters of the genitalia as given in table.

Genotype: *Heterocaria papuana* n. sp.

Heterocaria papuana new species (Plate II, Fig. 32)

Form orbicular, strongly convex. Head and pronotum very minutely, weakly punctured. Elytra more evidently punctured, the punctures very fine and rather close, with larger, more or less pellucid punctures interspersed, but these mostly restricted to the submarginal furrow. Head and prothorax testaceous yellow, the front and middle legs concolorous. Basal margin of pronotum narrowly black on middle third, and the lateral margins very narrowly blackish, except anteriorly. Thoracic epipleura with a yellowish white mark behind, this mark extending on to the fovea. Sternum black, but mesepimera yellowish white. Hind legs blackish, except tibiae, tarsi and inner side of femora, which are somewhat more brownish than front and middle legs. Venter dark brown. Scutellum and elytra black, the epipleura with a broad yellowish white streak on basal half. Disk of each elytron with eight testaceous yellow spots in four series: 3, 3, 1, 1. Spots of basal series largest, subequal in size, but differing in shape. Spot 1 oblique, nearly thrice as long as wide, situated between callus and margin. Spot 2 oval, except inner margin is nearly straight and anterior end pointed, placed just inside the callus. Spot 3 nearly circular and almost touching scutellum. Second series about half-way between the base and the middle of the length; the spots extremely unequal. Spot 4 subequal to 1, 2 and 3, transversely subquadrate and placed in the submarginal furrow. Spots 5 and 6 small and inconspicuous, 6 on one elytron being nearly obliterated. Spot 5 about equal distance from suture and lateral margin and distinctly posterior to 4 and 6. Spots 7 and 8 in longitudinal alignment with 1 and 4, the line distinctly diverging from outer margin posteriorly. Spot 7 placed just before the beginning of the last third of the length. Spot 8 subapical, a little closer to the suture than to the outer margin, and somewhat smaller than 7, which is approximately one-half as large as 1, 2, 3 or 4. Length and width, nearly 5 mm.

Described from 1 male (holotype), Laloki, Papua, July, 1909 (F. Muir), in collection of Hawaiian Sugar Planters' Experiment Station.

SPILOCARIA NEW GENUS

Form and structure much as in *Coelophora* Mulsant. Frons twice as wide as eye. Pronotum slightly foveate on each side behind, close to margin. Fovea of thoracic epipleura large, rather deep, oval, extending a little beyond middle. Prosternal carinae a little divergent behind, close together and parallel in front, where they extend a little beyond the middle of

prosternum. Mesosternum strongly, angularly notched in middle of anterior margin. Lateral margin of elytra somewhat explanate, especially on posterior half, but elytra without any pronounced submarginal furrow. Marginal bead of elytra slightly wider and more distinct than in *Coelophora*. Epipleura of elytra as in *Coelophora* except that the subhorizontal apical portion reaches quite to apex of elytron (in *Coelophora* beveled off a short distance before the apex). The minute punctures of pronotum a little closer and more distinct than those of the elytra. The larger, more or less pellucid punctures of elytra distributed about as in *Coelophora*. Characters of the male genitalia as given in preceding table.

Genotype: *Coclophora bissellata* Mulsant.

OENOPIA MULSANT

By original account *Oenopia* contained six species, of which two were placed in *Pania*, two in *Azya*, and two in *Oenopia*, s. str. Crotch in his Revision (1874) gave *addicta* as the type, but *addicta* was one of the species included under *Pania*. This seems to be an invalid type fixation, and I consequently suggest that *O. cinctella* Muls., should be considered as the type. *O. cinctella* was the first species included under *Ocnopia*, s. str.

PSYLLOBORA CHEVROLAT

Thea Mulsant should be included, as it shows no distinct departure from *Psyllobora*. The type of *Thea* has a somewhat different habitus from the American components, but in some of the other Old World species currently placed in *Thea*, the pattern of coloration is essentially the same as in American species.

Casey (1899) provided a table to separate the species of the United States, and the new species then erected have been unjustly merged in the Leng Catalogue as varieties of *P. 20-maculata* (Say). I here give a table of some of the North American species, mainly for the purpose of showing their relationship and specificity. This table is based on differences in the male genitalia.

KEY TO NORTH AMERICAN SPECIES OF PSYLLOBORA, BASED ON MALE GENITALIA

1. Siph elongate, tapering from before the middle into a very long, tenuous portion. . . . 2
Siph short, not tenuous at apex, although sometimes tapering to a needle-like point. . . 3
2. Median lobe of tegmen depressed, about six times as long as wide, a little expanded asymmetrically just before the apex, the margins then converging to an acute, slightly upturned point; paramera slender, a little shorter than median lobe and almost perfectly straight; siph somewhat longer than the body of insect, abruptly tenuous from the beginning of the second third. *20-maculata* (Say)
Median lobe of tegmen similar to preceding, but narrower, more aciculate, not expanded near the acute apex, which is not or just perceptibly upturned; paramera similar but more slender, slightly curved; siph practically the same. *tacdata* Leconte
3. Siph tapering to a needle-like point; median lobe of tegmen narrow and acicular. . . . 4
Siph compressed, becoming rather abruptly thinner and cylindrical near the beginning of the apical fifth and having a short membranous part at apex. 6
4. Siph very short, tapering rather abruptly in apical part to a fine acicular point. . . . 5
Siph somewhat longer, very gradually tapering from the middle to a very fine point; median lobe of tegmen some eight or nine times longer than wide, subdepressed, especially toward apex, which is acute and very slightly upturned; paramera elongate, almost as long as median lobe, slender, a little widened toward their bases and almost straight *parvinotata* Casey
5. Median lobe of tegmen finely acicular, cylindrical, tapering at apex into an acute rather strongly upturned point; paramera very slender, straight and somewhat shorter than median lobe *renifera* Casey

- Similar to preceding, but median lobe of tegmen less slender, compressed at base, slightly obtuse at apex, which is only slightly upturned; siphon a little longer. *juvenca* Timberlake
6. Paramera broad, more or less narrowed toward base, or rather slenderly clavate. 7
 Paramera moderately narrow, practically of the same width throughout, almost straight, and hardly more than three-fourths as long as median lobe of tegmen; latter about six times as long as wide, tapering nearly from the base to the acute apex, which is rather strongly curved upward. *luctuosa* Mulsant
7. Paramera sublamineate, not clavate. 8
 Paramera rather slender, nearly straight, except close to base and triangularly enlarged at apex; median lobe of tegmen about one-fourth longer than the paramera, with an elongate-oval, depressed, basal portion, tapering into a long slender upturned point, which is laminately compressed. *koebelci* Nunenmacher
8. Paramera narrowed about one-third toward base; median lobe of tegmen about one-fifth longer than the paramera and nearly of the same width, convex above, rather abruptly narrowed at beginning of apical fifth into a slender, strongly upturned point
borealis Casey
- Paramera somewhat shorter than in *borealis*, broader at apex and narrowed about one-half toward base; median lobe of tegmen about one-fifth longer than the paramera, shaped as in *borealis*, except that it tapers gradually to the acute apex, with a just perceptible constriction near beginning of the last fifth, the apex strongly upturned and distinctly laminately compressed. *deficiens* Casey

ILLEIS MULSANT

Illeis cincta of authors proves to be decidedly composite and I restrict the name to material from Ceylon, the type locality being "India orientalis." The segregates are extremely like *I. cincta* (Fabr.) and are not distinguishable with any certainty except by the male genitalia. Specimens of the *cincta* group have been seen from Los Baños, Luzon (Williams) and Java (Muir), but males are not available for study.

The following table, based mainly on the male genitalia, will serve to separate the known species of *Illeis*:

1. Elytra unicolorous testaceous, varying to creamy white and ferruginous. 2
 Elytra testaceous or yellow with sutural margins, transverse band at base, irregular band at middle, and apical mark, black; median lobe of tegmen moderately wide and convex above at base, ogivally narrowed at middle, with the apical half slender and slightly curved upward, the extreme apex being just perceptibly expanded and subtruncate; paramera ligulate, curved downward at apex, slightly longer than median lobe; siphon slender, cylindrical, slightly tapering but ending in a minute oval expansion (Australia). *galbula* (Mulsant)
2. Under parts of body entirely pale. 3
 Most of sternum, part of venter, coxae and femora, black; pronotum with two large black marks in the middle of the base, narrowly separated medially and reaching half-way to apical margin; median lobe of tegmen rather narrow, uniform in width to the rather abruptly upturned and truncate apex, as seen from above; in lateral view this lobe is considerably widened at base and thin at apex; paramera inserted close together, their bases somewhat laminately expanded and vertical, their apical portion slender, slightly curved downward; siphon slender, cylindrical, tapering to a fine point at apex (1 ♂, Deli, Sumatra, De Bussy). *bistigmosa* (Mulsant)
3. Pronotum with two small basal black marks. 4
 Pronotum entirely pale 8
4. Siphon not bifid at apex. 5
 Siphon subcylindrical, becoming a little slenderer and recurved in the apical sixth, the apex slightly thickened and rather deeply bifid; median lobe of tegmen rather stout, tubular, almost as broad as base of tegmen, tapering from the middle into a slender

- upturned point, paramera slender, almost straight except at base and slightly clavate at apex (Lahore, India).....*indica* n. sp.
5. Median lobe of tegmen moderately slender, more or less compressed at base and distinctly curved upward at apex, except in *cineta*..... 6
- Median lobe of tegmen very slender, acicular, compressed at base, almost perfectly straight; paramera as in *indica*, except that they are much more curved; siphon slender, cylindrical, gradually tapering from the base, so that the apex becomes about one-half as thick as the base (Japan, Formosa, Szechwan).....*koebeleri* Timberlake
6. Median lobe of tegmen having the apex strongly curved upward and more or less depressed 7
- Median lobe of tegmen nearly straight, strongly, laminately compressed, tapering into a very slender acute apex, the base a little deeper than broad; paramera slender, gently arched; siphon practically as in *confusa* (Ceylon).....*cineta* (Fabricius)
7. Median lobe of tegmen subacicular as seen from above, tapering at apex into an acute upturned point, its base strongly compressed, thrice as deep as wide, its dorsal outline strongly bisinuate (gently arched in basal half and concave in apical half); paramera slender, much as in *indica*, but longer and gently arched; siphon as in *indica* except that it is not quite so slender and simple at apex (Hongkong)
- confusa* Timberlake
- Median lobe of tegmen moderately stout, narrowed at beginning of apical sixth into an upturned point, compressed at base which is about twice as deep as wide, its dorsal outline arched in the basal third and gently concave beyond to apex; paramera shorter and stouter than in the preceding species, rather strongly curved near base, but otherwise straight; siphon much as in *confusa*, but longer (Chin-ling Mts., Shensi)
- shensiensis* n. sp.
8. Paramera stout, distinctly shorter than median lobe of tegmen; the latter broad, depressed, becoming narrower and subcompressed at base, about thrice as long as wide, the apex curved upward like the prow of a boat, with a short blunt projecting point; siphon about as in *confusa* (Amboina).....*amboinensis* n. sp.
- Paramera slender, perfectly straight and as long as median lobe of tegmen; the latter narrow, depressed, about four to five times as long as wide, and abruptly narrowed into an upturned point at apex; siphon nearly as in *amboinensis* (Luzon). *luzonica* n. sp.

***Illeis indica* new species**

Like *cineta*, but distinguishable by the male genitalia having the siphon bifid at apex, the median lobe of tegmen rather stout, convex above, about as deep at base as broad, and with a short slender upturned point at apex.

Described from 1 ♂, 4 ♀♀ (holotype male and paratypes), from Lahore, India. November 1910 (R. S. Woglum), in U. S. National Museum.

***Illeis shensiensis* new species**

Like *cineta*, but larger and differing in the male genitalia as shown in above table. Length, 5 to 6 mm.

Described from 2 ♂♂, 1 ♀ (holotype male and paratypes), from Chin-ling Mountains, Shensi Province, China, April-May, 1904 (Elliott Blackwelder), in U. S. National Museum.

***Illeis amboinensis* new species**

Like *cineta* except that it lacks the two basal black marks on the pronotum. Male genitalia as given in table. The paramera are comparatively short, straight, inserted close together, rather broad at base, sublaminar, tapering to the rather acute apex, and ciliate on their inner margin beyond the middle and at the apex. Length, 4 mm., width, 3.5 mm.

Described from 1 ♂ (holotype), Amboina, February 1908 (F. Muir), in collection of the Hawaiian Sugar Planters' Experiment Station.

Plate I.

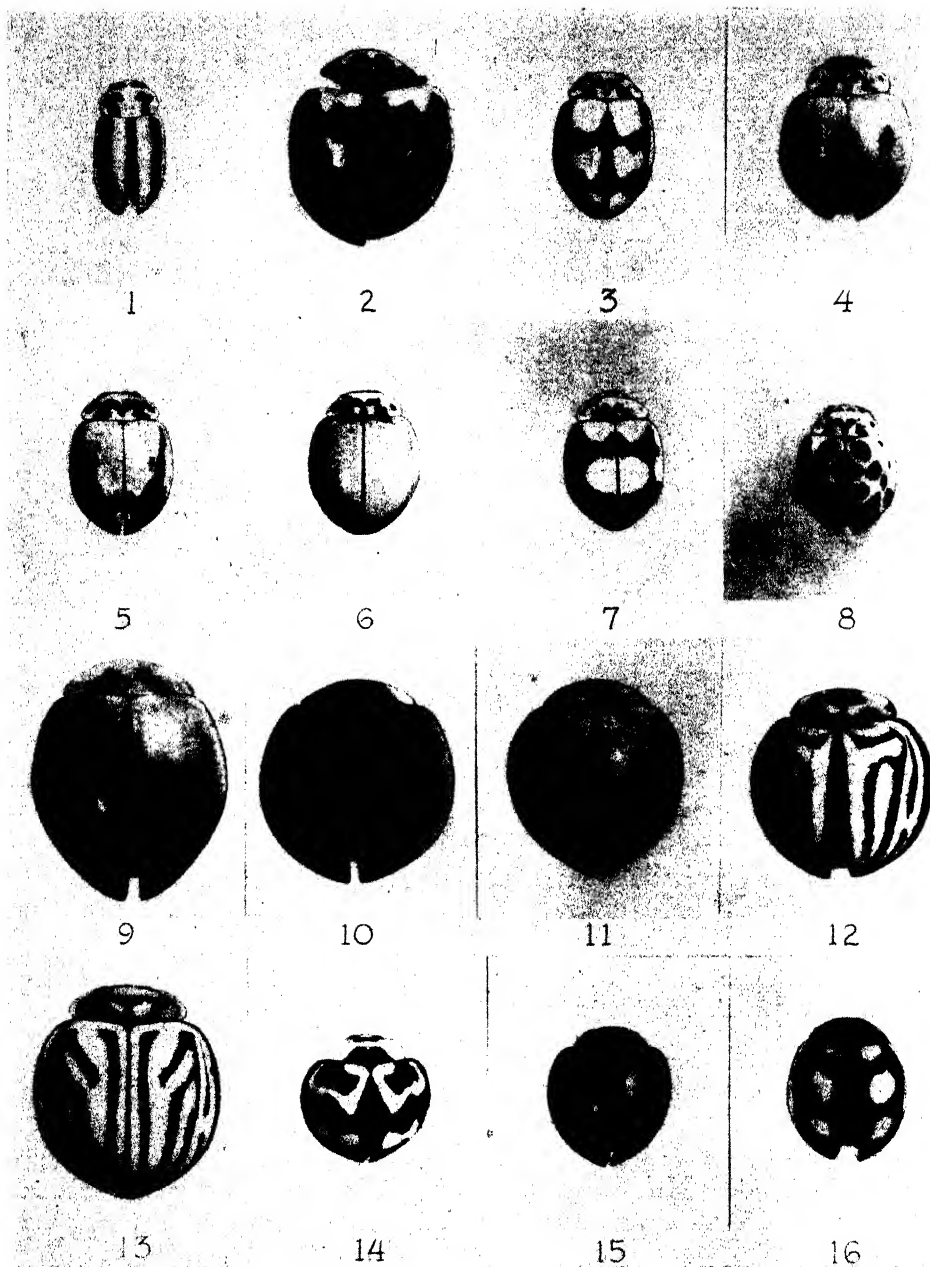


Fig. 1. *Macronaemia episcopalis*
 2. *Cissella furcifera*
 3. *Mulsantina picta minor*
 4. *Mulsantina picta minor*
 5. *Mulsantina picta minor*
 6. *Mulsantina mirifica*
 7. *Mulsantina mirifica*
 8. *Mulsantina mirifica*, var. *lynx*

Fig. 9. *Neomysia oblongoguttata*
 10. *Paraneda viridescens*
 11. *Egleis kingi*
 12. *Egleis delta*
 13. *Egleis edwardsii*
 14. *Egleis barronensis*
 15. *Verania flavovittata*
 16. *Protocaria scalaris*



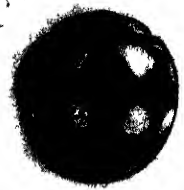
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Fig. 17. *Artemis circumusta*

- 18 *Bothrocavia pupillata*, var. *annectans*
- 19 *Phrynocaria gratiosa*, typical
- 20 *Phrynocaria gratiosa*, var. *flavoguttata*
- 21 *Phrynocaria gratiosa*, var. *palens*
- 22 *Phrynocaria gratiosa*, var. *nigrocincta*
- 23 *Phrynocaria gratiosa*, var. *nigrovittata*
- 24 *Phrynocaria gratiosa*, var. *koebeleri*

Fig. 25. *Eocaria muni*

- 26 *Gyrocaria guttata*
- 27 *Psyllobora koebeleri*
- 28 *Psyllobora guianica*
- 29 *Illeis galbula*
- 30 *Illeis koebeleri*
- 31 *Coccinellina ecuadorica*
- 32 *Heterocaria papuana*

NEW GENERA IN THIS PAPER

Coccinellina
 Paraneda
 Protocaria
 Phrynocaria
 Eocaria
 Gyrocaria
 Menochilus
 Pseudadonia
 Heterocaria
 Spilocaria
 Erythroneda
 Chloroneda

NEW SPECIES, SUBSPECIES, AND VARIETIES
IN THIS PAPER

Neomysia oblongoguttata caseyi
 Cycloneda polita flava
 Paraneda viridescens
 Protocaria scalaris
 Coelophora inaequalis comperei
 Bothrocalvia pupillata annectans
 Phrynocaria gratiosa flavoguttata
 Phrynocaria gratiosa palens
 Phrynocaria gratiosa nigrocincta
 Phrynocaria gratiosa koebelei
 Eocaria muiri
 Psyllobora juvenca
 Illeis amboinensis
 Illeis confusa
 Illeis indica
 Illeis koebelei
 Illeis luzonica
 Illeis shensiensis
 Coleomegilla maculata lengi
 Coleomegilla maculata tibialis
 Coleomegilla maculata boliviensis
 Coleomegilla maculata confluens
 Coleomegilla maculata maculata triangulifera
 Coccinellina ecuadorica
 Coccinellina shannoni
 Pseudadonia chiliana
 Heterocaria papuana

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Sugar Prices

96° CENTRIFUGALS FOR THE PERIOD
SEPTEMBER 16, 1942, TO DECEMBER 15, 1942

Date	Per pound	Per ton	Remarks
Sept. 16 - Dec. 15, 1942.....	3.74¢	\$74.80	Philippines

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No. 2

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Aconitic Acid, Calcium and Magnesium Aconitates in Sorgo and Sugar Cane Products

By H. A. COOK

The presence of aconitic acid in the juices of sugar-producing plants and in the products of sugar manufacture has been demonstrated qualitatively by many workers. Aconitic acid, its salts and derivatives have a commercial interest and value in the manufacture of plastics and plasticizers and they have been mentioned as having a bearing on some of the difficulties experienced from time to time in pan work and in the subsequent centrifugal separation of sugar crystals in sugar manufacture. A review of the available information on the subject is presented as a matter of local interest.

During a recent review of the literature in reference to problems and developments of particular importance to the sugar industry, a number of interesting references were found. Some of these references may have significant bearing on local problems in our own factories. Some of the references to research work showed revived interest in organic lime salts in sugar-bearing juices, particularly in aconitic acid and calcium and magnesium aconitates.

Aconitic acid, its salts and derivatives have a commercial interest and value in the manufacture of plastics and plasticizers and they have been mentioned as having a bearing on some of the difficulties experienced from time to time in pan work and in the subsequent centrifugal separation.

In view of these facts, the available information on the subject has been reviewed and brought up to date and is presented herewith as a matter of local interest.

The presence of aconitic acid in the juices of sugar-producing plants and in the products of sugar manufacture has been demonstrated qualitatively by many workers, but no very definite account of this compound in sugar juices or products has been taken until a recent publication by McCalip and Seibert (5) who made quantitative determinations of it in the sediment and scales of evaporators and pans and of its concentration in Louisiana final molasses. Behr (2) found it in molasses, in muscovado sugar, and in cane juice. Parsons (7) detected it in sorgum juice

from which, upon the addition of lime, the calcium salt separated on heating surfaces as a buff-colored, tenaciously adherent scale. Yoder (13) and Zerban (14) isolated aconitic acid from Louisiana sugar cane juice, and the former stated that it is the predominating organic acid in the juice. From the data he presented it may be calculated that he obtained approximately 0.3 per cent on the basis of dry solids. Taylor (10) found it in both healthy and diseased cane, and described the delicate color reaction it gives with acetic anhydride, a reaction which is characteristic, and was later modified by Furth and Herrmann (3). Both Taylor and Yoder noted that the calcium salt is less soluble in hot water than in cold. Prinsen Geerligs (8) found that a deposit centrifuged out of Cuban molasses contained a high percentage of calcium aconitate. Tanabe (9) recently found that aconitic acid accounted for 90 per cent of the total acid extracted with ether from a large quantity of juice from the cane variety POJ 2725. His recovery of aconitic acid may be calculated as roughly 0.1 per cent of the total solids. Nelson (6) isolated from Puerto Rican final molasses 0.8 per cent of aconitic acid, calculated on sample (equivalent to approximately 0.9 per cent on solids). Von Lippmann (11) found aconitic acid in sugar-beet products, and Beath (1) and others noted its occurrence in several species of native *Delphinium* and *Aconitum*.

Identification of the acid by these investigators was mainly through its lead, zinc, and silver salts. Reid (4) and coworkers described its p-nitrobenzyl and phenaacyl esters.

The first reference to actually accredit interference to pan and centrifugal operations to these compounds was in a report by Ventre (12):

In the present investigations, it was found that three constituents of the juices besides the sucrose content were directly concerned with its crystallization, *viz.*, starch, reducing sugars and salts of organic acids....

Some of the above prepared starch-free syrups were still found hard to work.

...crystals formed at a concentration below the degree of supersaturation necessary for sucrose crystallization. These crystals continued to grow until they reached a "smear" size and then stopped growing. When sucrose crystals were subsequently formed, these crystals remained as a "smear." They appeared to be lighter in density than the sucrose crystals as they came to the center of the centrifuge forming an impervious film preventing the massecuite from spinning.

...if the evaporator sirup was heated to 100° C. these crystals formed and readily settled to the bottom. After six to ten hours' time, as much as 10 pounds of this material per ton of cane could be separated of the consistency of wet sand. Analysis of this material indicated that it was mainly calcium aconitate with a small amount of magnesium aconitate.

The subject received extensive study by McCalip and Seibert (5):

The cream-colored sediment occurring in sirup and first and second molasses tanks during recent years in certain areas of Louisiana was studied and found to consist principally of calcium aconitate. The sediment was analyzed, and a method of separating aconitic acid from it and from related materials and of purifying it are described.

Refinery pan and evaporator scales were analyzed and found to contain aconitic acid. A simple test for the detection of aconitic acid in sediments and scales is described.

The aconitic acid content of syrups made without chemical clarification from juices from two different types of cane grown in different localities was determined and found to range from 0.75 to 1.33 per cent on solids. Two samples of Louisiana final molasses were analyzed and found to contain 1.80 and 2.52 per cent aconitic acid.

No reference or correlation was made between these results for aconitic acid and the workability of the syrups or molasses. However, they referred to the possibility that:

...sugar cane juice may prove a convenient natural source of aconitic acid, the industrial development and utilization of which has been limited because the most convenient source heretofore has been the dehydration of citric acid. The opening up of such a natural source will stimulate study of this acid, which is a derivative of both succinic and fumaric acids, and of its derivatives, many of which may readily be converted into maleic acid derivatives. Succinic and maleic acids and their derivatives are valuable intermediates in the manufacture of plastics and plasticizers.

H. P. Kortschak, of the Experiment Station staff, recently examined twenty samples of Hawaiian final molasses for insoluble crystals and reports as follows:

Twenty samples of molasses were examined microscopically for crystals insoluble in water. These were found in all but three. They were absent in one sample each from Ewa and Kohala. One Waimanalo sample was doubtful. Insoluble crystals were found in molasses from the following plantations: Honolulu Plantation, Hamakua, Honokaa, Kaiwika, Koloa, Lihue, Maui Agric., Paauhau, Waiānāe, Wailuku, Waimanalo, Waimānā, and Oahu.

A sample from Maui Agricultural Company contained 32 gms. of white crystals per liter separated with the super-centrifuge. Analysis showed them to consist mainly of a calcium salt of aconitic acid.

The presence of these compounds may possibly account to some extent for "scum" which is not infrequently observed in our centrifugals and at times is the cause of some difficulty in low-grade work. If it is present in our products in appreciable amounts at times, it may also have some commercial possibilities. This reopens a field of investigation that may be worthy of study.

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Depth of Planting Cane Affects Germination

By R. J. BORDEN

The depth of soil that is used in covering cane seed can influence not only the germination and establishment of the "stand" of cane but also the development of the crop during its early growth stages. Furthermore the results are not always similar when different depths of planting are used with different cane varieties or in different soils.

The accompanying set of photographs, taken at two, four, and six weeks after planting single-eyed seed pieces of three cane varieties at various depths in small pots of two different soils, offer visible evidence of the germination and initial growth differences which resulted from these several differentials, and suggest that these growth variations which are established early may be greatly magnified later on if the faster-starting stalks are in a position to shade out the slower ones. The data in Tables I and II support this photographic evidence.

The principal objective of this skirmish test (A-105—No. 159) was to note the effect on germination and the average rate of spindle emergence from cane seed covered by various depths of soil. Two soils were used: (1) an alluvial clay loam of nut-like structure from Makiki, and (2) a residual silty loam with an excellent granular structure from Manoa. Three cane varieties were included: H 109, 31-1389, and 32-8560.

Prime top-seed pieces were cut from variety plantings at Makiki which were comparable in age and condition. Careful selections of comparable single-eyed cuttings were subsequently made from these seed pieces and their ends were immediately dipped in Ceresan to protect them against rapid decay. After supplying both soils with phosphate, four of these cuttings were placed in each of eight small pots and covered either one inch, three inches, or five inches; thus a total of 32 eyes were planted for each treatment. All pots of soil were then wet to their field capacities and placed on cars which were run under glass shelter at night and during heavy rains.

Daily records were made of spindle emergence and from these the percentages of germination and the true average number of days for emergence were calculated. These data are summarized in Tables I and II.

TABLE I
PER CENT GERMINATION

Varieties	MAKIKI SOIL Depth of cover			MANOA SOIL Depth of cover			Variety average
	1"	3"	5"	1"	3"	5"	
H 109	81	84	0	97	88	60	68%
31-1389	100	95	44	100	95	100	88%
32-8560	100	97	50	100	100	50	83%
Average	94%	92%	31%	99%	94%	70%	
Average for soil:	Makiki = 72%			Manoa = 87%			
Average for depth of cover:	At 1" = 96%			At 3" = 93%		At 5" = 51%	

TABLE II
AVERAGE DAYS TO EMERGE FROM SOIL

Varieties	MAKIKI SOIL			MANOA SOIL			Variety average
	Depth of cover			Depth of cover			
	1"	3"	5"	1"	3"	5"	
H 109	11.5	17.6	10.6	15.8	16.8	14.5 days
31-1389	7.8	10.1	26.5	6.6	9.6	11.5	12.0 days
32-8560	10.0	12.7	19.5	8.6	11.0	18.0	13.3 days
Average	9.8	13.5	23.0	8.6	12.1	15.4	
Average for soil:	Makiki = 14.4 days			Manoa = 12.0 days			
Average for depth of cover:	1" = 9.2 days			3" = 12.8 days			5" = 18.5 days

Several facts seem to be quite clearly shown by these data in Tables I and II:

1. Except for the variety 31-1389 in Manoa soil, a covering of five inches of soil was responsible for a greatly reduced percentage germination. Differences between coverings of three inches and one inch were not highly significant.

2. Germination was significantly better in the Manoa soil. (Note: The Manoa soil has a more porous open structure which gives it better aeration than the Makiki soil.)

3. In all comparisons the 31-1389 seed gave a higher percentage germination than H 109 seed of comparable age and quality. Seed from 32-8560 germinated better than H 109 except when planted five inches deep in Manoa soil. Differences in the per cent germination between 31-1389 and 32-8560 were not significant except on Manoa soil with five inches of cover.

4. Depth of covering the seed had a very significant effect on the average number of days before the spindles emerged from the ground. Spindles from seed covered three inches appeared three days later than from seed planted one inch deep and when five inches of soil covered the seed, still another six days (for a total of 18 days) were required before they "broke ground." (Under wet soil conditions, rapid decay of the seed piece could take place within this average 18-day period.)

5. Seed of all varieties, regardless of depth of cover, germinated in fewer days when planted in the Manoa soil.

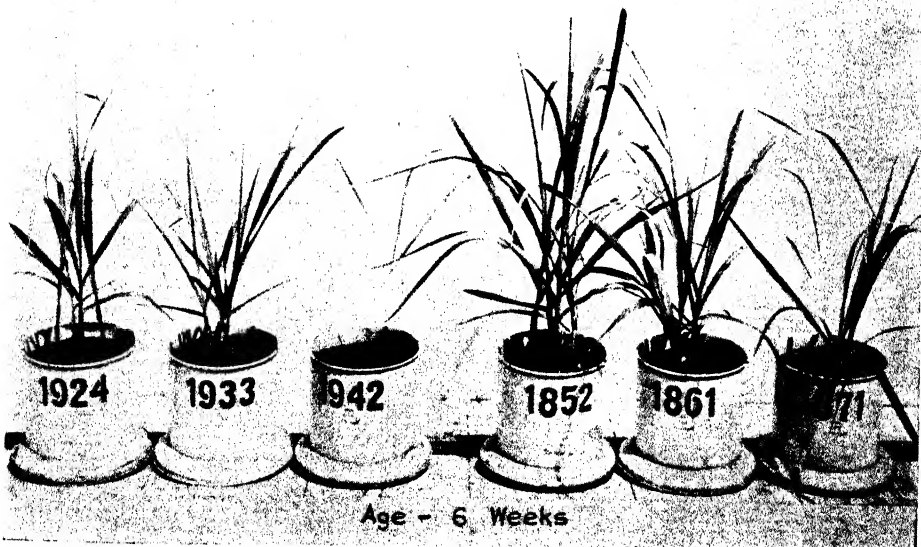
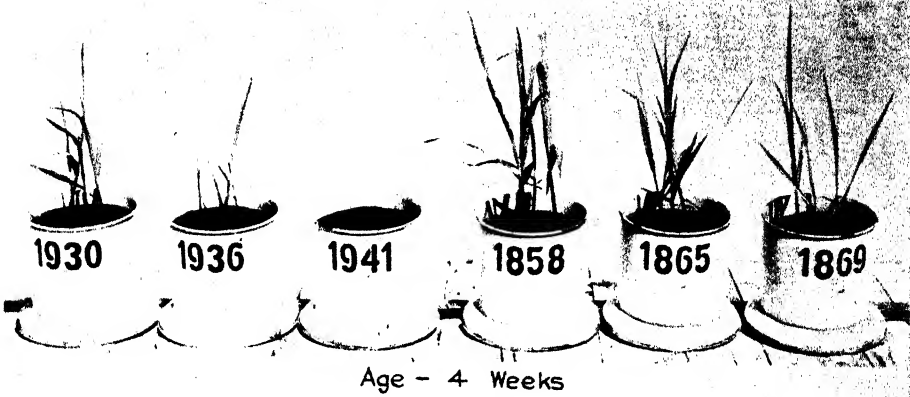
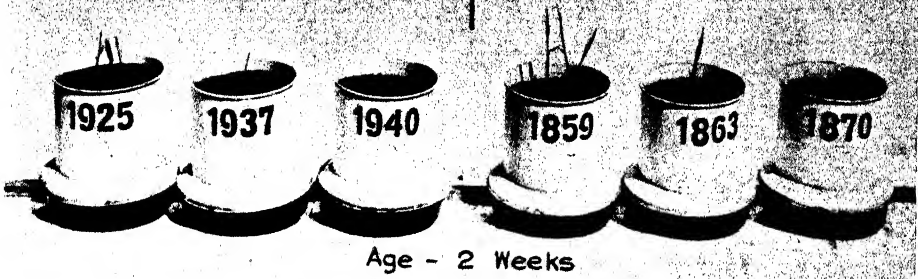
6. The 31-1389 shoots appeared earlier than 32-8560 except on Makiki soil when covered five inches. H 109 was slowest except on Manoa soil with five inches of cover. However, H 109 seed failed to germinate at all on Makiki soil when covered five inches.

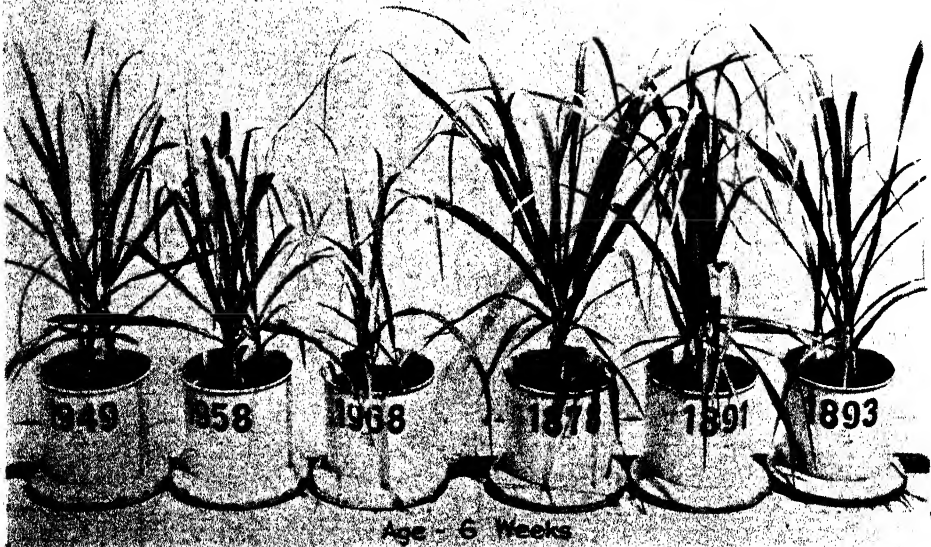
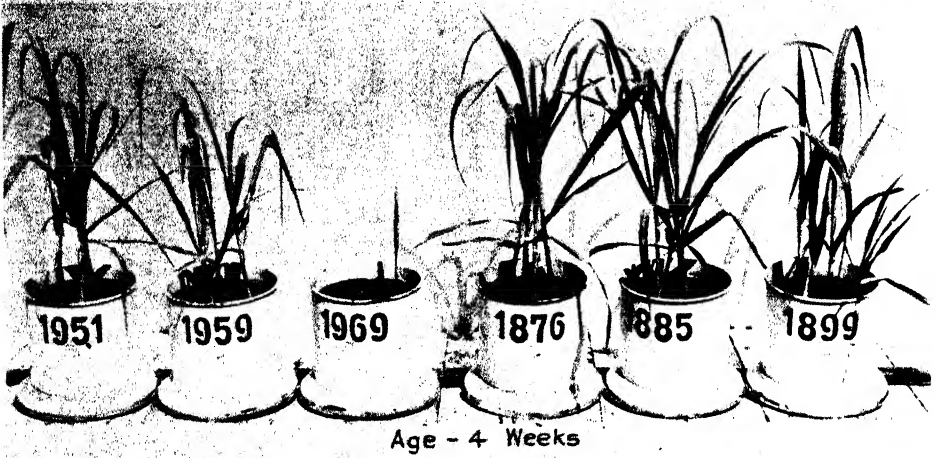
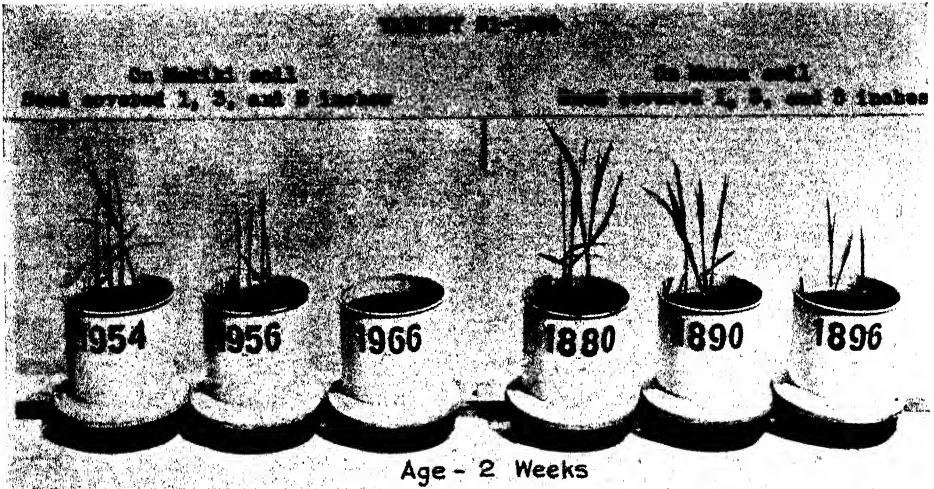
To sum up: the percentage of seed-piece eyes which germinated and the average number of days needed for their emergence was influenced by their depth of soil covering, and five inches of cover produced undependable and usually unsatisfactory results. Thus a uniform and shallow covering should give us best results when cane is planted on either irrigated or naturally moist soils.

VARIETY H 100

On Makiki soil
Seed covered 1, 3, and 5 inches

On Maunaloa soil
Seed covered 1, 3, and 5 inches

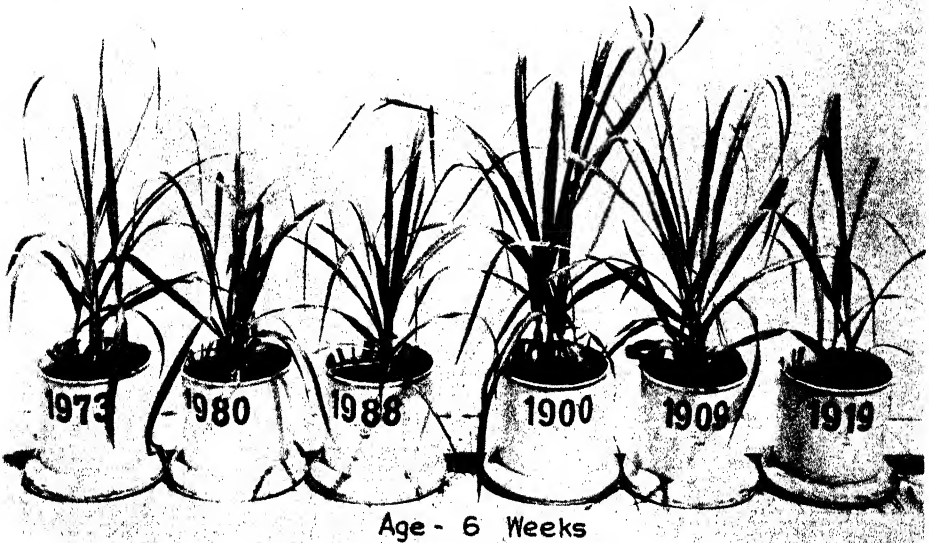
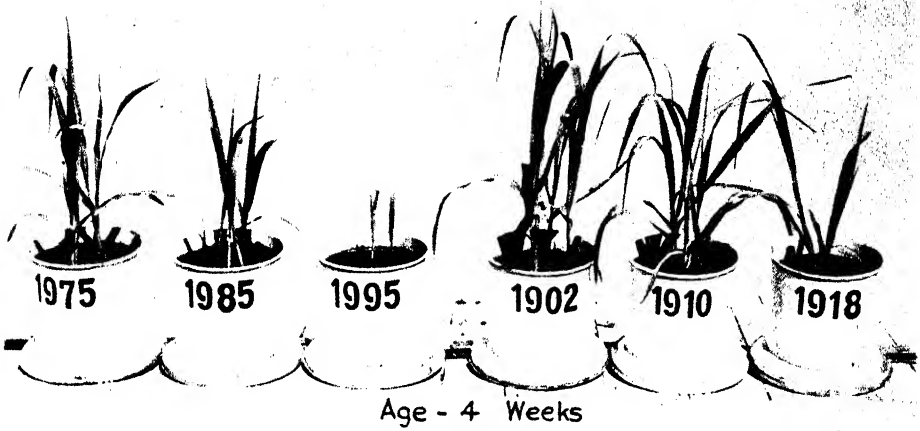
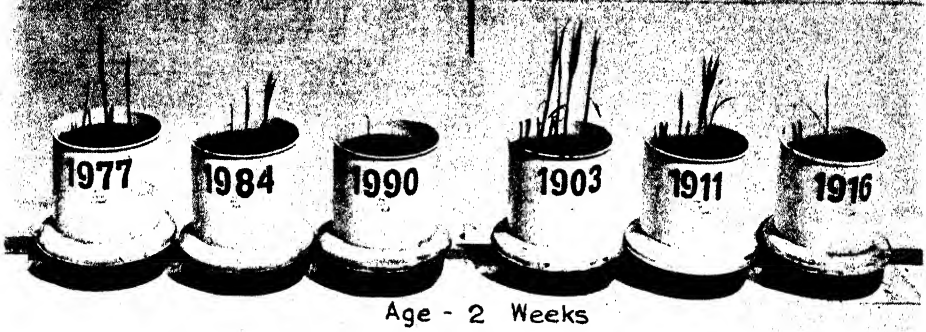




VARIETY 32-2560

On Makiki soil
Seed covered 1, 3, and 5 inches

On Maunaloa soil
Seed covered 1, 3, and 5 inches



A Recent Immigrant Tachinid Fly Parasite* of Noctuid Caterpillars in Hawaii

By R. H. VAN ZWALUWENBURG

The presence is noted of a recently arrived fly on Oahu which parasitizes a number of different kinds of destructive caterpillars, among them the corn-ear worm. In the laboratory it bred readily on the nutgrass armyworm also. The fly has a wide range in continental North America and in the West Indies, and evidently arrived here by chance within wormy tomatoes imported from Mexico. Its introduction was unsuccessfully attempted in 1923. The fly has an unusually brief life cycle, and the female, instead of laying eggs, deposits nearly mature maggots within the body of its victim.

In April 1942 a fly, new to the Islands and belonging to the family Tachinidae, was seen for the first time in the field on Oahu. C. E. Pemberton and the writer noticed considerable numbers of what were at first taken to be somewhat over-sized individuals of the sugar cane beetle borer parasite, in a potato field adjacent to cane at Waialua Agricultural Company. Closer examination of the specimens showed them to be identical with a specimen bred in Honolulu by Dr. F. X. Williams in March 1941 from a caterpillar in a tomato, probably from Mexico, purchased in the local market. Dr. Williams identified it as *Eucelatoria armigera* (Coquillett). Additional specimens were later found among unidentified material collected for introduction into Hawaii by H. T. Osborn from the state of Vera Cruz, Mexico, in 1923. Osborn's shipments were all dead upon arrival in Honolulu, due to the very short life cycle of the fly. It is presumed that the fly became established through the arrival of individuals in imported tomatoes, like the specimen which Dr. Williams intercepted. Incidentally this is a good example of how immigrant insects succeed in arriving here from distant places. Had *Eucelatoria* been an undesirable insect it would have been a successful evasion of plant quarantine precautions; that the species happens to be a welcome addition to the fauna is fortunate. It is not known, of course, how long this fly has been established here, but from its abundance when first found in the field, it may well have been several years. As suggested above, it somewhat resembles the *Ccromasia* parasite of the sugar cane beetle borer (see Fig. 1); however, it is slightly larger, measuring from about 6 to 8 mm., or $\frac{1}{4}$ to $\frac{1}{3}$ inch in length.

E. armigera was first described by Coquillett in 1889 (4, p. 332) from material bred from the corn-ear worm, *Heliothis armigera* (Hübner), in Los Angeles, California, under the name *Tachina* (*Masicera*) *armigera*. It has since been variously placed in the genera *Lydella*, *Blondelia*, *Anetia* and *Frontina*. Townsend in 1909 (8, p. 249) assigned it to a new genus, *Eucelatoria*.

Besides the corn-ear worm, this fly has several other hosts, mainly among the moth family Noctuidae. Osborn (6, p. 150) found *Cirphis latiuscula* (Herr.-Schff.) so heavily parasitized by what later proved to be this fly at El Potrero, Vera Cruz in July and August, that very few reached the adult stage. He says: "From the rapidity with which it overtook a threatened outbreak of the *Cirphis* it seemed

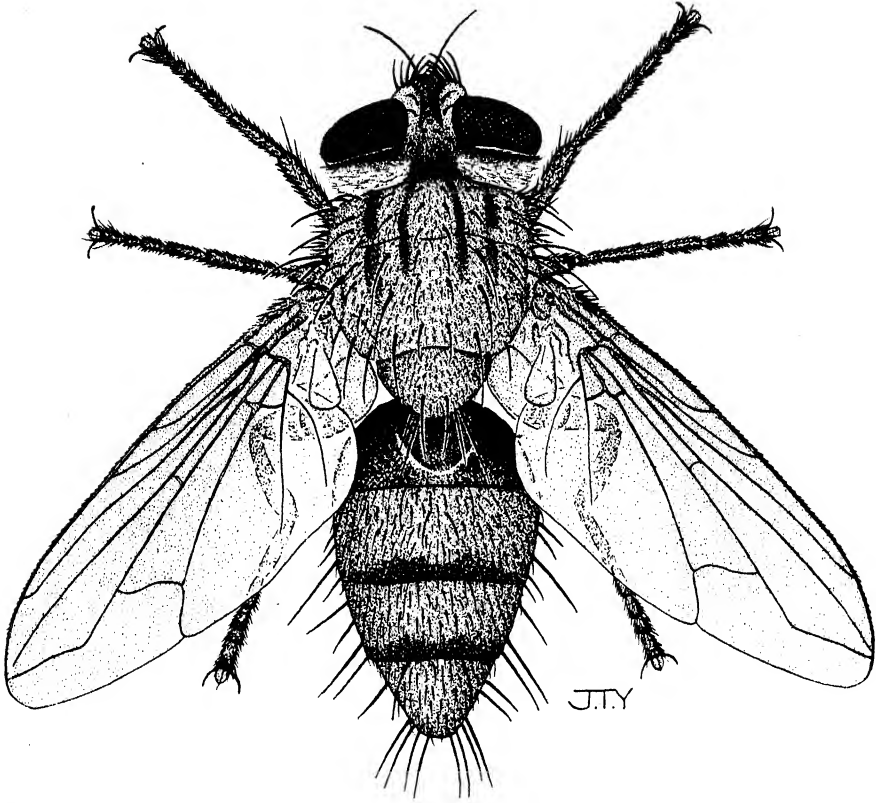


Fig. 1. Dorsal view of adult male *Eucelatoria armigera* (Coq.). (X12) (Drawn by J. T. Yamamoto.)

to me as possibly the most promising of the parasites observed on the armyworm in Mexico." Blanchard and Conger (2, p. 1065) bred this fly in California from larvae of *Prodenia praefica* Grote, an important pest of alfalfa and other field crops. Bruner (3, p. 96), crediting Cardin, records rearing *E. armigera* from *Laphygma frugiperda* (A. & S.) in Cuba. In May 1942 E. C. Zimmerman first reared it from the garden looper, *Plusia chalcites* (Esp.), feeding on foliage of *Spathoglottis* orchids in Honolulu. Subsequently it was found to parasitize this insect commonly; O. H. Swezey found that of 41 *Plusia* caterpillars collected at Ewa in December, about 36 per cent were parasitized by it. In the laboratory we have reared it without difficulty from half- to full-grown larvae of *Laphygma exempta* (Walker), the nutgrass armyworm, as well as from *Heliothis*. Attempts to induce parasitism of *Pieris rapae* (Linn.), cabbage butterfly larvae, were unsuccessful.

Greene's record (5, p. 43) of *E. armigera* bred from a sawfly larva, *Neodiprion* sp. in Georgia, shows that this fly does not entirely confine itself to moth caterpillars. Sawflies are members of the hymenopterous family Tenthredinidae; they are not represented in Hawaii. Wilson (11, p. 13) records a related fly, *Eucelatoria rubentris* (Coq.), parasitic in *Laphygma exigua* (Hübner) in Florida.

Because *E. armigera* has such a number of hosts among the generally destructive noctuids, it gives promise of considerable value under Hawaiian conditions. Should it take as readily to *Laphygma* in the open as it does in the laboratory, much may be

expected from it in control of the nutgrass armyworm. The fact that it attacks a species of *Prodenia* in California makes it reasonably certain that if *Prodenia litura* (Fab.), a general pest of major importance in the Pacific, should ever become established here, we shall have on hand a parasite to combat it.

E. armigera has the following wide range: California, Georgia, Florida, Texas (1, p. 943) and eastern Mexico on the mainland of North America, and Cuba and Puerto Rico (12, p. 353) in the West Indies. So far its establishment in Hawaii is certain only on Oahu, but small colonies have been sent to central Maui where the fly should become established with little difficulty.

Instead of laying eggs as most flies do, the female *Eucelatoria* deposits nearly mature larvae, or maggots, within the body of its victim, piercing the body with a highly specialized larvipositor (see Fig. 2). This mechanism (the "sternotheca" of Townsend) is a heavily chitinized, curved, thorn-like organ, widened toward the

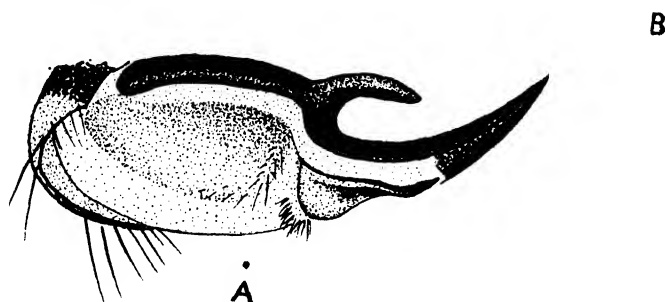


Fig. 2. Lateral view of larvipositor of female *E. armigera*; only a small portion of the lower curve (below a line drawn from A to B) is normally visible. For this illustration all the concealing structures have been removed. (Drawn by J. T. Yamamoto.)

base, and flattened and channelled on its outer curve. A small flap, or valve, overlies the channelled portion at the base, and the maggots are extruded from between this valve and the main part of the larvipositor. The process of piercing the integument of the caterpillar and depositing the maggot within takes place with extraordinary swiftness, and consists of a "flashlike dart at the host. The fly no sooner makes this dart than she is away again, yet during this brief fraction of a second the act of subcutaneous larviposition has been accomplished." (9, p. 90). When through feeding within the host, the maggot leaves the body of its victim and enters the soil to form a puparium, or pupal case. Caterpillars that have been pierced can usually be identified by the dried discoloration of the body fluids issuing from the puncture. Parasitized *Laphygma* larvae suffer complete collapse, sometimes within a day of being stung. *Heliothis* and *Plusia*, however, continue active and apparently normal for sometimes as much as five days after attack.

Townsend (7, p. 117) lists five different methods of reproduction among flies of this family: (1) host-oviposition; (2) leaf-oviposition; (3) supracutaneous host-larviposition; (4) subcutaneous host-larviposition; and (5) leaf-larviposition. With the advent of *Eucelatoria* we now have, among the seven tachinid flies in Hawaii, examples of four of these five methods, as well as still another in the case of *Ceromasia*:

<i>Frontina archippivora</i> Williston	Eggs laid on host
<i>Leucostoma aterrima</i> Williston	Eggs laid within host (coreid bugs of the genus <i>Corizus</i>)
<i>Leucostoma atra</i> Townsend	
<i>Chaetogaedia monticola</i> (Bigot)	Eggs laid on foliage, to be ingested by the host
<i>Eucelatoria armigera</i> (Coquillett)	Maggots deposited within host
<i>Archytas cirphis</i> Curran	Maggots deposited on foliage frequented by host
<i>Ceromasia sphenophori</i> (Villeneuve)	Maggots deposited loosely within tunnel of sugar cane beetle borer

The life cycle of *Eucelatoria* is very short, much shorter than that of any of the other species listed above, with the possible exception of *Leucostoma* on which little information is available. Even during the winter months *Eucelatoria* can complete a generation in less than three weeks. From larviposition to the emergence of flies of the next generation took from 12 to 13 days during January and February. Females were able to larviposit six days after emergence. Thus even in winter the entire life cycle takes from 18 to 19 days; during warmer parts of the year this may be expected to be somewhat reduced. Newly emerged flies mated within eight hours or less.

From 176 rearings of laboratory-bred *Eucelatoria* it appears that the proportion of sexes is very nearly equal: 86 males and 90 females.

	Reared Jan. 20–Feb. 7 Avg. mean temp. 72.9° F.		Reared Feb. 11–Mar. 7 Avg. mean temp. 69.1° F.	
	39 males	40 females	47 males	50 females
Days from larviposition to emergence of adult fly.....	12.0	12.2	12.9	13.2
Days spent by maggot within caterpillar	4.3	4.0	4.2	4.0
Days in puparium.....	7.6	8.2	8.6	9.2

Females required a slightly longer pupal period than males did. Males spent a somewhat longer time within the host than females; this feeding period was about the same irrespective of temperatures outside the host. However, the pupal period was prolonged by cooler temperatures, with a corresponding lengthening of the total period from larviposition to emergence.

The largest number of flies developing from a single caterpillar under field conditions was nine in the case of a *Plusia* larva. This number varies with the size of the host, and perhaps also with the number of flies present in the field and the number of caterpillars available. Concerning *Eucelatoria* parasitizing *Cirphis* in Mexico, Osborn (6, p. 150) says: "... several may develop in a single host. Occasionally up to five or six may be obtained, although many caterpillars have only one, and the average [is] probably not over three or four." If a larva is exposed to too many flies, super-parasitism may result, that is, more maggots (14 in the case of one small *Plusia* larva) are deposited than can develop. The results of breeding with *Laphygma* larvae in the laboratory were as follows:

- 31 caterpillars (Jan. 20–Feb. 7) produced 39 males and 40 females, or 2.5 flies per larva.
 34 caterpillars (Feb. 11–Mar. 7) produced 109 puparia (3.2 per larva) from which issued 47 males and 50 females, or 2.8 flies per larva.*

As a rule the more flies developed per larva the smaller their size, although the size of the host also is a factor, of course. Flies from a five-puparia lot bred from a

* To make observation easier, no soil was present in the containers in which the maggots pupated; had puparia been formed under more nearly natural conditions the percentage of successful emergences might have been higher.

last-stage corn-ear worm were larger than those from a lot of equal number reared from a last-stage nutgrass armyworm. Of 32 *Laphygma* larvae:

7 produced 1 fly puparium each
 8 produced 2 fly puparia each
 6 produced 3 fly puparia each
 1 produced 4 fly puparia each
 8 produced 5 fly puparia each
 2 produced 8 fly puparia each

L. R. Smith of our Agricultural department has examined the following data and found them to have statistical significance. They show an inverse ratio between the number of *Eucelatoria* maggots in a single larva, and the length of time spent by the maggots within the host:

No. of flies produced per host larva	Days spent within host larva
1 (7 examples)	5.4
2 (8 ")	5.0
3 (6 ")	5.0
4 (1 ")	4.0
5 (8 ")	3.5
8 (2 ")	3.2

Nearly mature maggots were several times observed to extrude the caudal end of the body through a break in the skin of the host. After some minutes thus exposed they would retreat into the host again. The maneuver suggests a method of respiration, the respiratory spiracles being situated on the posterior end of the maggot's body.

Under laboratory conditions, fed on brown sugar and water, the maximum length of life of male *Eucelatoria* was between 17 and 18 days. Reproductive females lived a maximum of from 17 to 18 days, while one female, presumably mated, but sterile, lived for 30 days.

The maximum productiveness noted was 42 puparia each in the case of two females; the potential productiveness is certainly much higher. According to Townsend (10, p. 49) *Eucelatoria* has a capacity of 100 to 200 maggots. Of 11 females confined with males and having almost daily access to caterpillars for larviposition, only three reproduced in the laboratory:

Female B—lived from 17 to 18 days; when 12 days old parasitized a *Laphygma* larva, the only one successfully parasitized by this female; one puparium and a male fly resulted.

Female C—lived from 15 to 16 days; first parasitized two of three *Laphygma* offered, when six days old; parasitized ten *Laphygma* and one *Heliothis* on nine different days, the last when the fly was 14 days old; five *Laphygma* offered it were not attacked. This fly produced 42 puparia from which 16 males and 20 females issued.

Female E—lived from 13 to 14 days; first parasitized two of three *Laphygma* offered, when six days old; parasitized one *Heliothis* and nine *Laphygma* on seven different days, the last when the fly was 12 days old; two *Laphygma* offered it were not attacked. This individual produced 42 puparia from which 19 males and 19 females issued.

Several of the apparently sterile females stung *Laphygma* larvae so severely that they soon showed the collapse typical of parasitized individuals, but no flies resulted.

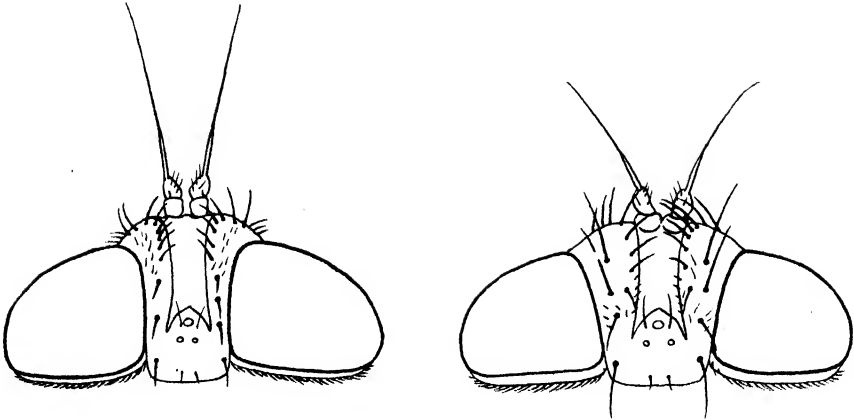


Fig. 3. Dorsal view of head of *E. armigera* (male, left; female, right) showing comparative width of the interocular space and differences in setation. (Drawn by J. T. Yamamoto.)

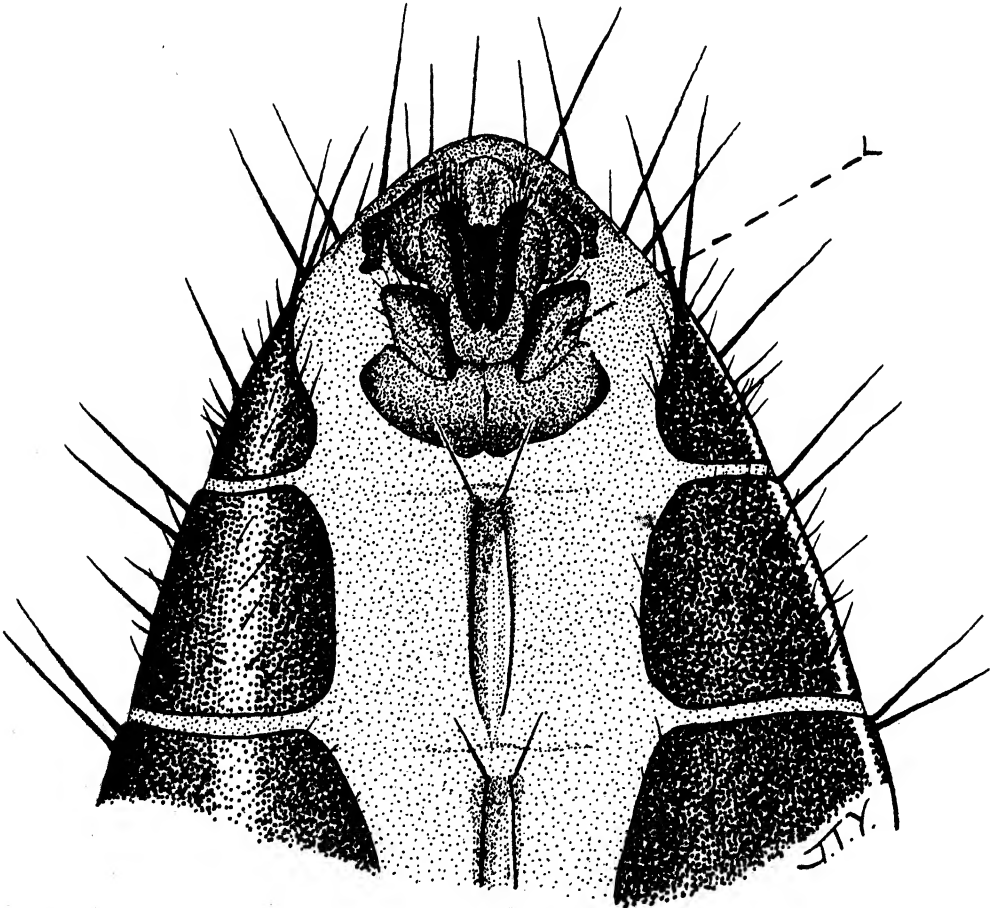


Fig. 4. Ventral view of abdomen of male *E. armigera*. In this drawing the plates concealing the male hypopygium are purposely separated to show the fifth sternite with its lobes (L) which distinguish the male from the female. Normally only the tips of these lobes are visible. (Drawn by J. T. Yamamoto.)

As early as the fifth day after emergence female flies showed interest in *Laphygma*, and a few flies of that age attacked larvae. It is doubtful if maggots were actually deposited by five-day-old females; no reproduction was obtained in such instances.

Townsend (10, p. 49) states that the uterus of *Eucelatoria* is "in 3 or 4 coils or loops with the eggs and maggots obliquely on end in single or double file. . . ." Examination of fresh material showed the eggs at the upper end of the tubes to be obliquely placed at about a 45-degree angle; as they progress downward the eggs develop into maggots, which gradually assume a longitudinal position with the caudal end downward. The eggs as described by Townsend (9, p. 88) are "stout subcylindric, rather elongate, slightly tapered at ends, chorion membranous and transparent." The maggots are "stout subcylindric, white, with more or less complete transverse spine rows, the spines more numerous on ventral surface and the fourth segment more extensively spined than the others, the last segment tipped dorsally with 3 short, stout, hooked spines."

The sex of *Eucelatoria* flies can be distinguished with little difficulty: (1) the space between the eyes (see Fig. 3) is relatively wider in the female than in the male, and the setae, or hairs, on the upper part of the front are arranged in a double row in the female, in a single row in the male; (2) the intermediate segments of the underside of the female have, along the middle line, a strongly spined carina, or ridge; this is absent in the male, which has two more or less rounded, readily visible lobes on the posterior margin of the fifth ventral segment (see Fig. 4).

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The Production of Sorgo Sirup and Sugar

By H. A. Cook

Sorgo cane is considered to possess very desirable agronomical characteristics; it matures very quickly and can be grown over wide areas in the United States. Crystallization of sugar from sorgo juices has been a very difficult problem and much research has been conducted toward that end for many years. During recent years research carried on largely under the guidance and cooperation of the Carbohydrate Unit of the Agricultural Chemistry Division, Bureau of Agricultural Chemistry and Engineering, United States Department of Agriculture, has been responsible for the development of a process whereby the crystallization of sucrose has been effected. This has revived considerable interest in the sorgo plant on the mainland. A review of this work is presented.

During recent years interest in the production of sorgo sirup and the manufacture of sugar from sorgo juices has been revived on the mainland. Under present conditions it appears that this interest may become more active; we therefore present an outline of some of the developments which have taken place, especially in regard to the crystallization of sucrose (cane sugar) from sorgo juices. The research was carried on largely under the guidance and cooperation of the Carbohydrate Unit of the Agricultural Chemistry Division, Bureau of Agricultural Chemistry and Engineering, United States Department of Agriculture.

Prior to 1933, reports from the U. S. Department of Agriculture refer to the poor or non-uniform quality of sorgo sirup produced on many farms in the United States. A recent report by E. K. Ventre of the above bureau summarizes the early situation in *The Sugar Journal*, Vol. 3, December 1940, p. 23, as follows:

The crystallization of sucrose from the juices of sorgo plant is a project that has had much intensive study in the past. The previous investigators succeeded in demonstrating the facts that the plant has very desirable agronomical characteristics and sucrose content, which compared very favorably with those of sugarcane and sugarbeets at that time. However, due to the failure to develop a workable process for the extraction of sucrose from the juices the investigations were abandoned.

In a similar report made to the Chief of the Bureau of Agricultural Chemistry and Engineering, U.S.D.A., 1940, pp. 28-29, is the following statement:

Utilization of suitable varieties of the sorgo plant for the production of sugar and its byproducts is a possibility that may be of great importance under some circumstances, and renewed attention has been given to this problem. It now appears that the difficulties which previously handicapped this development may be overcome. Sorgo matures in about 4 months and can be grown over wide areas in the United States. In certain sections it could be used to supplement sugarcane and sugarbeets, making possible a longer and more profitable period of operation of existing cane- and beet-sugar factories and providing a new and profitable crop to growers.

The lack of uniformity in the quality of sirup, sedimentation occurring in the sirup, and the inability to precipitate sucrose from the juices was a real problem in the sorgo-producing areas of the United States. The report of the Chief of the Bureau of Chemistry and Soils of the Department of Agriculture, 1935, p. 7, summarized the situation as follows:

This defect has been a serious handicap to farmers in obtaining the fullest profit from this important cash crop. Investigations were continued for the purpose of devising means whereby sorgo sirup of more uniform and improved quality may be made directly on the farm.

A continuation of the report states:

One of the principal difficulties experienced by sorgo sirup producers is slow boiling, which often results in scorching the sirup. This trouble was found to be caused by the presence of starch in the juice, which in addition to retarding evaporation will, if present in large quantity, cause actual jellying of the sirup.

A practical method was devised for use of malt extract in the production of sorgo sirup for the elimination of defects such as "jellying," excess turbidity, and difficulty in concentrating the sirup to the required density, which result from the gelatinization of starch contained in the juice. This solves a difficulty which has in the past been the cause of much poor-quality sorgo sirup.

The report continues:

The use of a high diastatic malt for hydrolyzing the starch is recommended to overcome this difficulty. It was found that the greatest benefit from the use of malt is obtained by applying it after the juice has been evaporated to a semisirup. The usefulness of the diastase method for preventing slow boiling, scorching, and jellying of sorgo sirup was demonstrated in cooperative work with the Arkansas Agricultural Experiment Station. The drought of 1934 apparently was responsible for the fact that it was practically impossible to produce sirup of satisfactory quality by the usual farm methods. By employing the newly developed improved method, sirup of excellent quality was produced from the same sorgo cane.

Work on this subject was continued in 1936 along with studies relating to the factors of ripeness of the cane and topping the cane. This is summarized in the Report of the Chief of the Bureau of Chemistry and Soils, U.S.D.A., 1936, p. 5.

In an effort to eliminate the difficulties which tend to prevent the production of good sorgo sirup, an investigation was made of the quality and composition of sirups prepared from different portions of the sorgo stalk at different stages of maturity. This work was done in cooperation with the Mississippi Agricultural Experiment Station, and four of the best varieties of sorgo grown in northern Mississippi were studied. The data obtained show that the quality of sorgo sirup can be greatly improved by using cane which is ripe but not overripe, as determined by the condition of the seed heads, and also by discarding a certain proportion of the cane tops. The improvement in quality and possible increase in value may offset the comparatively small loss in yield of sirup, particularly when the forage value of the tops is considered. Most of the starch, which causes "jellying" and makes it impossible to boil down the sirup to required density without scorching, is present in the upper part of the stalk and can be eliminated by topping. A study of the sucrose and reducing sugar relationship in different parts of the stalk also revealed that it might be practical to establish a practice of selecting a certain portion of the sorgo stalk for sirup production, whereby sucrose and dextrose crystallization in the sirup, which detracts from its value, could be avoided without incurring too great loss in yield of sirup. Objectionable sharp flavor or "tang," which is correlated with the titratable acidity, can probably be minimized by variety selection, avoiding overripe stage of maturity, and topping to the fifth internode.

Studies in cooperation with the Mississippi Agricultural Experiment Station continued and were incorporated in the Report of the Chief of the Bureau of Chemistry and Soils, 1937, p. 5.

The malt diastase process for the prevention of jellying and consequent slow boiling or scorching and the practice of topping the stalks to the fifth internode to reduce the mineral, acid, and starch content of the juice fully meet the limitations of farm-scale sirup production. Studies conducted... during the year showed that further improvement in quality of sirup could be accomplished by combining these two methods.

Cooperative work in Mississippi on the composition of juices and quality of sirup from different sections of the stalks, in which 8 additional varieties of sorgo cane were used and about 100 samples of sirup were made, confirmed the conclusions from last year's work that the farm value and marketability of sorgo sirup can be materially improved by cutting off and discarding several joints of the stalk from the top. The discarded top joints can be well utilized as feed for livestock, and the comparatively small reduction in yield of sirup is more than compensated by the improvement in quality of the sirup and the feeding value of the discarded top sections of the stalks.

Farmers' Bulletin 1791, *Farm Production of Sorgo Sirup*, by C. F. Walton, Jr., E. K. Ventre and S. Byall, was published in 1938 and contains a very complete treatise on the manufacture of sorgo sirup on a small farm scale. This bulletin is based upon and embodies the results of the preceding work on this subject. It covers the topping and milling of the cane; location, layout and size of mill and equipment; treating and evaporating the juice; removing sediment from the sirup; treatment with diastase; how to prevent sugaring; problems and methods of canning, marketing, etc. The juice is extracted with a small two- or three-roller mill, passing the cane through twice, and obtaining an extraction of 55 to 68 per cent. This juice may be centrifuged to remove a large amount of the starch, or merely neutralized with lime and heated. After clarification it is boiled to a semisirup. The semisirup is treated with diastase malt to hydrolyze the starch, and settled for a period of six hours to remove sediment. They state (p. 34) that dextrose crystallization is controllable by blending of varieties of cane or of sirup:

Success in preventing crystallization of both dextrose and cane sugar nevertheless depends upon having these sugars present in the sirup in the right proportions.

The proportions usually satisfactory are about equal amounts. They suggest also (p. 34) the use of invertase:

Another method of preventing cane-sugar crystallization, if variety selection, harvesting at the proper stage of maturity, and low topping fail to give good results, is the "invertase process." This is a practicable process in which an extract of yeast (invertase) is used during the manufacture of the sirup. The yeast extract converts a portion of the cane sugar into the two sugars dextrose and levulose, so that the resulting more properly balanced sugar content of the sirup will not deposit either cane sugar or dextrose crystals. The invertase process, of course, should not be used when the trouble to be remedied is due to dextrose.

It is disclosed by these comments that difficulties were encountered through crystallization occurring in the sirup due to three forms of sugar, *i.e.*, sucrose, dextrose and levulose. These crystallize in the sirup under different conditions, depending somewhat upon their proportions in the sirup.

The subject of topping the stalks of the sorgo plant received further study in connection with the crystallization of sucrose and dextrose in sorgo sirups. E. K. Ventre and S. Byall further reported in *Distribution and Variation with Maturity of Dissolved Solids, Sucrose, and Titratable Acidity in the Sorgo Stalk*, Journal of Agricultural Research (1937), Vol. 55, pp. 553-562. They state on page 562:

If sorgo juices are to be used for the crystallization of sucrose, previously recommended topping practices are incorrect. The reverse procedure should be used, that is, the bottom internodes should be discarded, as they have relatively a much lower coefficient of purity. In some cases several internodes at the bottom of the stalk are much below the practical crystallization limits for sucrose.

In the manufacture of sirup from the sorgo stalk, however, topping, or discarding the three or four upper internodes, reduces the tendency of the sirup to crystallize sucrose and also produces sirup with a minimum of acidity or sharp "tang."

The above report makes the first reference encountered since 1933 concerning the commercial possibilities of sucrose crystallization from the sorgo sirup.

Correlation of starch content, jellying, crystallization, topping and maturity of the sorgo stalk were further studied and reported upon by E. K. Ventre, S. Byall, and C. F. Walton in 1939, *Jellying and Crystallization of Sirups Made from Different Parts of the Sorgo Stalk at Different Stages of Maturity*, Journal of Agricultural Research (1939), Vol. 59, pp. 139-150, and were summarized on page 149 as follows:

The starch content and jellying of sorgo sirups are correlated and increase with maturity of the sorgo. The upper portions of the stalk produce sirups higher in starch content. The number of parts of the stalk yielding sirups that jelly increases with maturity.

Sucrose crystallization occurs most frequently in sirups made from the upper part of the sorgo stalk. The number of parts of the sorgo stalk yielding sirups from which sucrose crystallizes increases with maturity.

Dextrose crystallization occurs most frequently in sirups made from the lower portions of the stalk. The number of portions of the stalk yielding sirups from which dextrose crystallizes decreases with maturity.

We have three reports along the same line on the same subject during 1940. One of these, also by C. F. Walton, E. K. Ventre and S. Byall, *Effects of Low Topping and Diastatic Malt Extract on Composition and Quality of Sorgo Sirup* Journal of Agricultural Research, Vol. 60 (1940), pp. 427-432, gives the following summary on page 432:

Most of the sirups with a starch content of 1.25 percent or higher, jellied or became extremely viscous, whereas those made by the use of high-diastatic malt extract had a starch content considerably under 1.0 percent, in many cases only about 0.25 percent, and did not jelly.

The ash content of the samples varied considerably with the variety but it was usually higher in sirups made from the top portion of the stalk.

In making sirups with juice from the tops alone, it was observed that scorching usually occurred before evaporation to the standard density of sirup was completed. This is characteristic of juices of high starch content, and accounts for the dark color and strong flavor of some sirups.

The topped stalks consistently produced sirup of better quality than the whole stalks, which, in turn, gave better sirup than did the tops alone.

Relatively little improvement in quality resulted from simply allowing the semisirup to settle, without malt-extract treatment, before completing the evaporation.

All sirups produced by the process in which high-diastatic malt extract was used were of better color, flavor, and clarity than the sirups made from corresponding parts of the stalk by the standard procedure.

The results show that sirups of the highest quality are produced by using starch-hydrolyzing enzymes to supplement reasonably good topping practice.

The report of the Chief of the Bureau of Agricultural Chemistry and Engineering, 1940, pp. 28-29, mentions the fact that the work had progressed so far that the primary *crystallization of sucrose could be readily accomplished*. Excerpts from the report follow:

Analyses were also made of the objectionable sediment formed to different extents in samples of sirup from various sources, in order to obtain information on the nature and origin of this sediment which may lead to the adoption of preventive or remedial measures. Farm-made sirups free from sediment would have improved marketing possibilities.

Preliminary results obtained in experiments on a pilot-plant scale at Meridian, Miss., field station showed that starch and its degradation products in sorgo juices are among the most objectionable constituents that prevent efficient crystallization of sugar. Means for the re-

removal of interfering starch and starch products by physical methods and by diastatic conversion were devised, with the result that primary crystallization of sucrose could be readily accomplished. Satisfactory analytical methods were developed for determining sucrose, dextrose, and levulose in sorgo juices. The results of a study of the diurnal variations in the sucrose, dextrose, and levulose contents of the sorgo stalk were not indicative that the synthesis of sucrose and starch occurs in the stalk juice. Promising results, from the standpoint of quality, were obtained in laboratory studies on the production of refined sugar in the form of sirup ("liquid sugar") from sorgo juice by the use of "carbonaceous" ion-exchange materials. Best results were obtained when the juices were passed through the cation and anion exchange materials alternately. Excessive wash water, however, was required to remove color after regeneration of the anion exchange material which is a serious objection. An investigation was begun on methods for the preparation of an ion-exchange material from fibrous materials impregnated with aniline dyes.

Some interesting developments are brought out in the above report, including the commercial possibilities in the crystallization of sucrose (cane sugar) through the development of efficient means for removal of starch and its deleterious products; that materials which are ordinarily contained in the sediment of the semi-sirup may be the cause of considerable interference to free boiling in the pans and subsequent centrifugal separation; appreciable further benefits may be derived from carbonaceous ion-exchangers in further clarification of the juice, together with studies toward making fibrous ion-exchangers impregnated with aniline dyes.

E. K. Ventre also described the work and the results obtained in a pilot-scale plant toward the crystallization of sucrose from the sorgo juices in *Preliminary Report of the Crystallization of Sucrose from Juices of Sorgo Plant*, The Sugar Journal, Vol. 3, No. 7 (1940), pp. 23-30, excerpts from which follow:

In the present investigations, it was found that three constituents of the juices besides the sucrose content were directly concerned with its crystallization, viz., starch, reducing sugars and salts of organic acids, each of which can best be discussed separately.

Starch Content of the Juices—Influence and Methods of Removal:

[There is shown] a wide variation in the starch content of the juices; these variations are due mainly to varietal characteristics but in some instances are, in part, due to the degree of maturity of the cane. It has been shown in a previous work that the starch content and sucrose content of the juices increase with maturity. The juices of the plant contain mature starch and intermediates from various stages in the process of synthesis which do not lend themselves to physical separation and require the use of enzymes for their conversion.

Physical Separation of Starch:

The first method employed for physical separation of the starch was liming the juices to neutrality, heating to boiling point in an open defecator, thereby raising the starch to the top of the defecator and drawing off the clear juice from under the "blanket" of scums. Reference... will show that this method is effective to the extent of removing an average of 70.92% of the starch originally present in the juice.

The second method studied for the physical removal of starch from the juice was by tabling the raw juice. ... When followed by lime and heat defecation we find... an average removal of 80.80% of the starch originally present in the juice. The tabling process had the disadvantages of requiring a large installation due to a low tabling rate and permitted deterioration of the juices besides a considerable juice loss when the tables were drained.

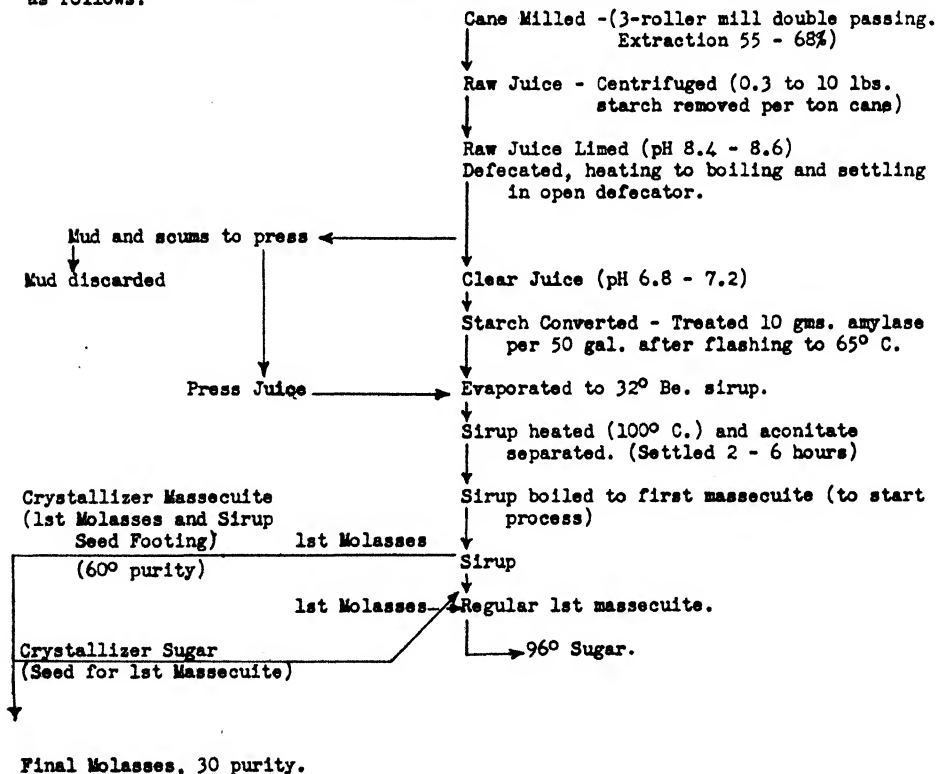
The third method studied for the physical removal of starch from the juice was centrifuging in a solid basket centrifuge. ... this method alone removed an average 70.72% of the starch originally present in the juice and in combination with defecation removed 92.57% of the starch originally present in the juice. This method possessed, in addition, the advantages of no deterioration hazard due to the rapid separation, and the starch removed was in a compact form more suitable for use as a by-product. Varying with the variety, it is shown... that from 0.3 to 9.85 pounds of starch per ton of cane may be recovered as a by-product by this method.

Enzyme Treatment for Removal of Residual Starch and Intermediates:

The influence of starch on the crystallization of the sirups under vacuum is first noted by the sirups becoming gelatinous and not circulating at a concentration below that necessary for securing the degree of supersaturation required for crystal growth. This does not involve a large amount of starch; for example, at 88% solids only 12 percent of water is present and in sirups having as much as .40% starch on solids the starch percent water would be 3.5% sufficient to cause a jell at this concentration. Further concentration of the starch obtains in the molasses and from this it is readily evident that nearly complete starch removal is necessary for free working of the juices in the factory. ...it was found necessary subsequent to physical separation to use enzymes for the final removal of starch and its products. The first enzyme used was an infusion of ground malted barley. The use of this material as an enzyme source was successful insofar as securing crystallization but it possessed the disadvantages of

PROCESS USED FOR SORGO PILOT PLANT OPERATION

The process that gave the most satisfactory operation may be diagrammed as follows:



E. K. Ventre, Preliminary Report of the Crystallization of Sucrose From Juices of Sorgo Plant. Contribution No. 163, Carbohydrate Unit, Agric. Chem. Research Div., Bureau of Agric. Chem. & Eng., U.S.D.A.

An adaptation of chart shown in The Sugar Journal, Vol. 3, No. 7, p. 25 (1940).

requiring an amount of infusion which added a considerable amount of reducing sugars to the sirup, required a pH below that optimum for the clarification and gave a low conversion to monosaccharides. However, this is one of the cheapest sources of starch-converting enzymes and due to this fact this material will be the subject of further study.

Pancreatic amylase was found to possess the advantages of working at the pH of optimum clarification, gave a high conversion to monosaccharides, and added no reducing sugars of itself to the sirup. In the use of either enzyme it was found most convenient to draw the hot defecated juice into a vacuum evaporator flash to cool to 65° C. and add the enzyme being careful to maintain a temperature below 70° C. during the time required for the enzyme to convert the starch. It was found that for sirup so prepared to boil satisfactorily that it show neither starch nor dextrin test with iodine. Using these methods and present equipment it was found that it required 40 grams per ton of cane of this material to get a working sirup. Further work on this should permit a reduction in the enzyme demand.

Calcium Salts or Organic Acids [Salts of Organic Acids—Aconitates]:

Starch free sirups prepared by a combination of the physical and enzymatic methods were found to be difficult to work for the reason that when they were heated and concentrated in the vacuum pan crystals formed at a concentration below the degree of supersaturation necessary for sucrose crystallization. These crystals continued to grow until they reached a "smear" size and then stopped growing. When sucrose crystals were subsequently formed, these crystals remained as a "smear." They appeared to be lighter in density than the sucrose crystals as they came to the center of the centrifuge forming an impervious film preventing the massecuite from spinning.

It was found that if the evaporator sirup was heated to 100° C. these crystals formed and readily settled to the bottom. After six to ten hours' time, as much as 10 pounds of this material per ton of cane could be separated of the consistency of wet sand. Analysis of this material indicated that it was mainly calcium aconitate with a small amount of magnesium aconitate. It was found possible to further remove only a small amount of this material from first and second molasses made from sirups previously treated.

[It was shown] that the ash removal for sirup treated by heat and settling from six varieties to be a nearly constant figure of 0.72% ash on solids. Calculations based on these figures show an average ash removal of 1.545 pounds per ton of cane.

On the subject of *Reducing Sugars and Their Effect on Crystallization*, a tentative formula is presented for calculating the amount of crystallization of sucrose in relation to invert sugars.

It is interesting to note that thirty purity molasses may be produced up to a reducing sugar (as invert sugar) content percent solids of 13.20%.

Again it is desirable to emphasize that this paper must be considered as a preview of the work and the success obtained shall serve only to prove that utilization of the sorgho plant is not impossible and reopen the field of experimentation with this plant.

Notes on the Manufacture of Cane Sugar Syrups, Invert Sugar Syrup, Candy and Similar Products

(Prepared by the Sugar Technology Department of the Experiment Station)

The demand for candy, table and cooking syrups and molasses has increased tremendously in Hawaii along with the large increase in civilian and service population during the war. At the same time shipping space is at a premium. It should be possible to meet most, if not all, of this demand with local sugar and other products and thereby increase profits as well as reduce shipping space requirements between Hawaii and the mainland under war conditions. A resumé has been prepared touching upon the possibilities of manufacturing these products locally.

There does not appear to be any real reason or any great obstacle that should keep the Territory of Hawaii from being entirely self-dependent, in so far as products to satisfy its sweet tooth are concerned. Hawaii is an important source of sugar, both to ourselves and to the mainland of the United States. Sugar and the various products, which are or can be made from it, are a very important adjunct in the diet of all of our population. In one form or another it enters into a large share of the items of food which we consume. Many industries and many products depend upon sugar in one form or another; it is used in table and cooking syrups, molasses and invert sugar syrup, candy, bakery goods, beverages and many similar products.

Some products require sugar in its ordinary form as refined sugar, which is practically pure sucrose or as a pure sucrose syrup which is sometimes termed "neutral syrup." Other products require a mixture of sucrose and glucose in the form of a syrup. While glucose, which is a mixture of dextrose and levulose, occurs naturally to some extent in cane juices, it can readily be produced or its concentration increased in a sucrose-bearing syrup by one of several available inversion processes by which one molecule of sucrose becomes two molecules of glucose, or one each of dextrose and levulose. Still other products require the use of straight glucose or invert sugar syrup in which all sugars are in the form of dextrose and levulose. All of these ingredients to meet the requirements can be produced from local sugar and in conjunction with local sugar factories.

During the past years and even at the present time many tons of candy, table and cooking syrups and molasses, invert syrup or glucose and also yeast products from molasses have been manufactured on the mainland from sugar which could well be the identical sugar shipped over there from Hawaii and then these products shipped back into the Territory. This takes up valuable shipping space especially on the return voyage when this shipping space can, under present conditions particularly, be used for other essential needs.

Table syrups, cooking syrups and molasses can be made from raw cane juice evaporator syrup, raw, or washed raw sugars. Invert syrup or glucose can be made from raw, washed raw, or refined sugar. Candy can be made directly from the

same materials. To prepare the above items it requires but an inverting agent, such as a suitable acid or invertase to convert the sucrose or a portion of it in our products to glucose. Invertase can be prepared locally from ordinary yeast and it has already been demonstrated that yeast of a very high quality can be produced from our final molasses. This locally produced yeast could take care not only of the invertase needs, but also of the needs of the bakery industry in the Islands. A sufficient quantity could also be produced to supply materially the protein needs in food for humans and for livestock. As an acid-inverting agent large quantities of citric acid are already manufactured and are available from the pineapple industry, or if necessary it could be made from final molasses.

Thus we find that all of the necessary ingredients—crusher juice, evaporator syrup, raw, washed raw, or refined sugar, and final molasses—are available in large quantities to start production of all of the necessary saccharine products required by the confectionery, bakery, and beverage trades of the Islands. It appears that the production of these items for local needs is a logical step for the sugar industry. In most cases the additional equipment required would not be great, since practically all of the necessary equipment in one form or another is on hand at the factories.

Considerable work along this line has been carried on by the Sugar Technology Department on a laboratory scale and a fairly extensive search of the literature has been made. Instructions and formulas for various classes of candies and syrups from raw sugar syrup, raw sugar, washed raw sugar, and refined sugar have been prepared and were issued in the Department's Activities Report No. 4 of August 10, 1942. The candies include fudges, hard candies and brittles. It is assumed that most of the commercial quantities and qualities of candies will be taken care of by the new factory now being considered by the Hawaiian Pineapple Company.

The list of syrups includes table and cooking syrups and molasses, glucose or invert sugar syrup and "Hawaiian Sugar Honey." Considerable quantities of table syrup are now being produced by Honolulu Plantation Company, and several other plantations including Ewa, Oahu, and Hawaiian Commercial have for some time been making syrups for their employees or for sale through their plantation stores. These procedures, formulas, and additional data are presented herewith for general information.

The flavor and quality of these products will depend upon the care used and the method employed in their production and upon the initial product from which they are made. The desirability of each will depend upon individual tastes and the nature of the product for which it is to be used. Cooking, table, and invert syrups made from crusher juice or evaporator syrup will have a darker color and a stronger taste than those made from raw sugar and will also contain more of the mineral content and organic nonsugars. Raw sugar will in turn produce syrup with darker color and a stronger taste than washed raw or refined sugar. The color and flavor may be varied almost at will by the proper selection or blending of the starting materials. A light-colored, high-quality glucose or invert syrup would presumably be started from washed raw or from refined sugar or refinery syrup.

Discussion of various points in connection with these products and their manufacture will be undertaken in the following pages.

SYRUPS FROM HAWAIIAN SUGARS

Syrup of excellent quality for table or cooking purposes can be produced locally provided containers can be secured for its storage and distribution. This syrup can be produced from cane sugar products by the use of either invertase or citric acid. Invertase or invertase yeast can be prepared locally. Citric acid is already produced in quantity by the local pineapple industry.

In general the flavor of table syrup made from washed raw sugar is more pleasing to the majority than that made from straight raw sugar, although if the syrup made from raw sugar is clarified somewhat by heating and settling or by filtration, the flavor is very similar to that made from washed sugar. There is a fairly large demand and sale for syrup made from crusher juice or evaporator syrup throughout the Territory.

Methods of making these plantation syrups differ and so do the flavor and color of the products. The flavor and color depend upon a number of factors in addition to the character of the materials from which the syrup is started and the method of making the syrup. If raw juice or crushed juice is used as the starting point, the juice is clarified by one of several methods, each of which may have some effect upon the final color and flavor.

At this point it may be of interest to cite some comments by H. S. Paine from the U. S. Department of Agriculture, Bulletin 1370, 1925, pp. 69-72:

The quality of flavor most desired in cane sirup is smoothness, with enough of the typical cane-juice flavor to give to it the unmistakable taste of the cane. Although the production of much "caramel" flavor during evaporation of juice to sirup is to be avoided, a little may improve the general character of cane sirup by making sweetness more noticeable and masking less desirable flavors. Cane sirup possesses more flavor than may at first be apparent. While this can be measured only by tasting, a method based upon the detection of flavor at various dilutions with water shows that in the average sirup the flavor is from 25 to 60 per cent more persistent than sweetness. The formation of small quantities of caramel and partial neutralization of the acidity of the sirup tend to equalize the intensity of sweetness and flavor.

Cane sirup owes its food value essentially to its sugar content.

The salts (ash constituents) and organic nonsugars in cane sirup have some incidental food value. Recent studies in nutrition and dietetics indicate that sugar-cane sirup and molasses contain a noteworthy quantity of vitamins. As the dietary value of the nonsugar substances is somewhat indefinite, however, the food value is usually calculated in terms of energy units on the basis of total sugars. Taking as a basis of calculation a fuel value of 3.749 large calories per gram for invert sugar and 3.955 large calories per gram for cane sugar, the energy value of one pound of cane sirup of the average composition . . . would be 1,188.6 large calories. One gallon of such sirup of [about 72° Brix] . . . weighing 11.35 pounds per gallon, has a food value of 13,491 large calories.

Sugar cane syrup if evaporated to a moderately high density will crystallize unless a portion of the sucrose has been inverted, while on the other hand a thin syrup is very likely to ferment in warm weather. Invert sugar does not readily crystallize. As the proportion of invert sugar to sucrose is increased, the likelihood of the syrup crystallizing is lessened. The problem is to make a syrup that will not crystallize when evaporated to a water content of about 20 per cent. Satisfactory syrups have been made in our laboratory having a density range of 72-78° Brix. Transformation of sucrose into invert sugar on a commercial scale can be brought about in several ways, but only those of commercial importance will be discussed. The two important procedures, inversion by heating with an acid and by

the action of an enzyme obtained from yeast called "invertase," will be discussed in some detail in the following sections.

Inversion Methods:

The methods employed for the conversion of the sucrose to glucose can be modified in their application and the degree of inversion can be controlled to meet the particular need. A system of control and adherence to certain details is essential to the success of the method employed and to obtain a high quality in the desired product. As stated above, two general methods are employed for inversion on a commercial scale; (1) heating with acids, and (2) the use of invertase. They will be discussed in their general aspects, including laboratory experience and comments gleaned from the literature on the subject, and then their application given in greater detail in reference to their use for specified products.

(1) *Inversion by Heating With Acids:* This method has been employed extensively. Hydrochloric or sulfuric are the acids most commonly used for the purpose, although tartaric, phosphoric and citric acids have been employed. All but the last of these are difficult to obtain in sufficiently pure form in the Territory at the present time as they are usually imported. Citric acid is produced locally and can be obtained in sufficient quantity for most purposes. Citric acid is very effective for the purpose, but as with all acids precautions must be observed in its use. The solubility of ordinary iron and copper is quite high in the presence of citric acid at the reactions and temperatures which are necessary. However, the amount of iron dissolved during the boiling has no noticeable effect upon the taste of certain syrups, and iron containers could be used for the inversion under some conditions. The amount of copper dissolved would affect the syrup or other product considerably and precludes the use of this metal until excess acidity is neutralized.

Some of the commercial methods employed using acids are described in the literature and may be of interest.

F. A. Lopez Ferrer describes a method which he uses in making invert syrup direct from cane. His article has been abstracted in *Facts About Sugar*, March 1936, p. 105, as follows:

The new industry of invert sugar manufacture has been of much value to certain mills in Cuba, as it has led to the utilization of cane that could not be used for the manufacture of crystal sugar, and in some cases has prolonged the grinding season 60 to 90 days. So far, sixteen Cuban factories have been making this product, and in 1935 they produced 54,468,283 gallons of invert syrup. The author is of the opinion that with continuance of restriction on sugar output, the amount of syrup that can be marketed at a good price is about 60,000,000 gallons.

In the process of manufacture supervised by the author, the operations are the same as for the production of crystal sugar up to the point of obtaining the thick juice (meladura), with the sole exception that the juice is kept as acid as possible, for the sake of economy in the inversion. No more lime is used in the juice than is required to produce a pH of 5.8 or 6.0, which corresponds to an inversion of 3 or 4 per cent when the juice is warmed up to more than 100° C. in the heaters and evaporators. The thick juice at 54° to 60° Brix is subjected to inversion with sulphuric acid in the proportion of 0.85 to 1.0 gallon of acid per 1,000 gallons of juice, which means 1.75 to 2.0 gallons of acid per 1,000 gallons of final invert syrup. Mixture of acid and thick juice is effected in six tanks provided with steam coils and compressed air. The mixing and heating must be done gradually and thoroughly to avoid caramelization. The first operation is called "active inversion"; it lasts 50 to 60 minutes and results in a syrup of 30 to 45 purity.

In the next operation, called "inversion in repose," the mixture is maintained at rest at a temperature corresponding to the working temperature in a vacuum pan. The thick juice is then concentrated under vacuum to 85° or 87° Brix. Before being discharged from the pan, the syrup is treated with one gallon of milk of lime (12° to 15° Baumé) per hectoliter of pan capacity, so that the pH on discharging will be about 6.8.

The author considers that the ideal invert syrup should contain 25 to 30 per cent real sucrose, and from 48 to 53 per cent glucose, which will give approximately 78 per cent of total sugars. The three factors of acid, time and temperature must be adjusted so as to secure maximum factory economy.

Incrustation of heating surfaces is more troublesome than in regular sugar manufacture, and metal surfaces should be treated with acid-resistant paint wherever possible, in order to diminish corrosion.

Another article on the production of inverted molasses in Cuba by D. G. Aurioles has been abstracted in *Facts About Sugar*, October 1937, p. 402, as follows:

The manufacture of inverted molasses (or invert syrups) though simple, requires select equipment and careful supervision. The technique of the process varies slightly according to the type of syrup used but always in agreement with the following rules: The Brix of the syrup varies from 72° to 84°, polarization after inversion from 20° to 42°. As an inverting agent, pure hydrochloric acid of 22° Baumé is used where the finest product is desired; the time of inversion varies from 20 to 40 minutes in the case of high purity syrups to 2 hours with inferior sugars. The final pH of the inverted syrup varies from 4 to 4.5, except when some customers wish a pH of 5.5 to 6.6, in which case the adjustment is made with milk of lime. In some mills phosphoric has been substituted for hydrochloric acid. Iron should not be used in the equipment, or the color of the product will be impaired.

In some cases, as in the preparation of a lower grade product, concentrated sulphuric acid is used for the inversion at the rate of 0.30 lbs. per 100 lbs. of syrup. The mixture is heated up to 200° F. and kept at this temperature until a rapid analysis shows a polarization of 0.10°; then milk of lime is added sufficient to bring the pH to 6.0. The syrup is then pumped to "waiting tanks" of the vacuum pans to be concentrated to 80°-85° Brix.

Another method which constitutes a practically continuous inversion and concentration process is described by J. R. Osuna in an abstract in *Facts About Sugar*, December 1940, p. 40, as follows:

The system devised by the author aims at effecting operations with as much convenience as possible. The process requires a heater, three cylindrical tanks, one or more vacuum pans, and a cooler. For the last, use may be made of a heater in which the cold water is circulated through the steam coils. The three tanks are connected with each other by 8" piping and are used in rotation for inversion and mixing of the syrup.

The syrups are continuously concentrated in the vacuum pans, which are fed from the bottom; the concentrated syrup is continuously withdrawn from the pan by a pipe, which opens a foot above the top plate of the calandria and which is prolonged downward to a piston pump on the main floor of the factory. When the first or 'A' tank is one-fourth full, running in of the inverting agent (acid or yeast) is begun and it flows along with the syrup until 'A' is full. The filling of tank 'B' is then begun; half the contents of 'A' are transferred to tank 'C,' and when 'B' is full its contents are divided between 'A' and 'C,' which are discharged in rotation after completing the scheduled inversion time cycle.

A similar procedure is described by M. A. Mascaro and is abstracted in *Facts About Sugar*, April 1940, pp. 31-32, as follows:

Heretofore the procedure of making high test molasses (concentrated and acid-inverted cane juice) has consisted of mixing and heating the syrup and acid in a tank provided with a stirring apparatus and transferring the mixture to another tank for completion of the inversion cycle. To counteract the slowing down of the inversion process as the mass cools, it has been necessary to reheat it in the same tank by means of steam coils.

Experience has shown that this stationary heating in large tanks is detrimental because there is a loss of total sugars by caramelization and decomposition. For this reason the author has introduced a system which he describes as follows:

The juice follows the usual course until it reaches the quadruple effect, but is defecated with a minimum of lime, or with no lime at all if circumstances admit. The concentrated syrup (40°–42° Brix) is received in a tank for mixing with dilute acid in the usual manner. Then the mixture is pumped through the first of three small heaters of high speed design supplied with exhaust steam, where it is brought to 195°–200° F., and is discharged into tanks arranged in series. The acidified syrup remains in the tanks until its temperature falls to about 175° F., at which temperature the rate of inversion becomes too slow. The juice is then pumped through the second heater, to be again raised to the temperature at which inversion proceeds rapidly, and is discharged into a second series of tanks (or old crystallizers) where it remains until the purity has fallen to the point desired. The third heater is kept as a spare and for use during cleaning periods.

Considerable loss is avoided by this process. Even mills that obtain 79 per cent of total sugars may now obtain 80 to 81 per cent and perhaps more.

The California & Hawaiian Sugar Refining Corporation at Crockett produces an invert syrup for the beverage trade which demands a high-grade product. We have been informed that they use No. 1 concentrated liquor, which is one of the highest quality liquors, and that hydrochloric acid is used for the inversion. The requisite amount of acid is mixed with a definite amount of water in a rubber-lined tank. Inversion takes place in a glass-lined tank. A given amount of the liquor is run into the latter tank and the mixture of acid and water is added to this while the liquor is being agitated. This mixture is then gradually heated until a temperature of about 85° C. is reached and this temperature is maintained until the desired degree of inversion is accomplished. The resulting invert syrup is then brought to approximately the neutral point with caustic soda. The inversion of about 2,000 gallons of syrup can be accomplished in about an hour and a half with about 5½ pounds of hydrochloric acid.

Inversion can be accomplished in a similar manner with citric acid. It appears to require a slightly longer time to accomplish the inversion with citric than with hydrochloric acid. The required time will vary somewhat, depending upon the purity of the make-up syrup, lower purities requiring a longer time. If a particularly light-colored invert syrup is not required, the inversion can be carried out in iron tanks using any desired syrup, such as evaporator syrup or syrup made up from raw or washed raw sugar. The density should be in the neighborhood of 65° Brix and the inversion temperature about 85° C. As soon as inversion is carried to the desired point, the syrup is neutralized to about pH 6.2 with sodium bicarbonate or sodium carbonate. After the addition of the necessary amount of soda to neutralize excess acidity, the final concentration may be carried out in copper utensils. If a very light-colored invert syrup is required, the nature of the make-up syrup can be varied as well as the inversion procedure. Details of the inversion method will be described in a later section in connection with the various products discussed. On the basis of laboratory tests a thousand pounds of sugar, representing approximately 140 gallons of syrup at 65° Brix, would require about 9½ pounds of citric acid and 10½ pounds of sodium bicarbonate to give slightly over 110 gallons of invert syrup at 82° Brix.

(2) *Inversion with Invertase:* The use of invertase or a highly active invertase yeast is largely supplanting acids for the purpose of inverting sucrose to glu-

cose in the manufacture of table and invert syrups. It is generally conceded that invertase produces a better quality syrup with a better taste than the acid method and there is less corrosion of equipment. It is possible to produce in the laboratory a satisfactory invertase from yeast and there are several methods available for the production of high invertase-content yeast which can be produced in connection with any yeast plant. Some of the references, for the production of highly active invertase yeast, invertase and the use of invertase for syrup production, may be of interest at this point.

The use of invertase in connection with high-test invert molasses is commented upon by F. Guerrero, Proc. 13th Ann. Conf. Asoc. Tecnicos Azucareros, Cuba, 1939:

The invert molasses industry has entered a new phase with the introduction of a yeast with powerful inverting properties, which enable the manufacturer to dispense with the use of acids that destroy sugar values and corrode the equipment.

A process used in Cuba is described by J. C. Gonzalez Maiz and his article has been abstracted in *Facts About Sugar*, March 1941, p. 30, a part of which follows:

The process consists of delivering the sterilized syrup at the proper Brix and temperature to a vessel provided with arrangements for stirring and heating with steam. The required amount of yeast is added, and inversion is allowed to proceed until a polarization test shows zero, or better, minus 8 to minus 9 polarity. Then the syrup is again sterilized at 80° to 90° C. and concentrated in a vacuum pan to 85° Brix. The distillers, who are the principal consumers of inverted molasses, require a product that has been approximately 70 per cent inverted.

With a Brix of 58°, a temperature of 58° C., and an inversion cycle of 12 hours the amount of invertase yeast consumed is 3.93 per cent on sucrose in syrup.

A process by which the yeast used for inverting syrup can be prepared at a factory is reported by A. P. Fowler and abstracted in *Sugar*, June 1941, pp. 37-38, as follows:

It is generally conceded that inversion of sugar cane syrups by the use of yeast invertase is better than by the use of sulphuric or other acid. In the original Guerrero process, yeast for the purpose is propagated from pure cultures at the factory. An alternative is to purchase the yeast from manufacturers who specialize in its production on a large scale. The author offers a factory process as a middle course between the pure culture technique and purchase from outside sources. In this process 1500 gallons of a 6.5° Brix solution of well sterilized defecated juice is adjusted to a pH of 4.5 by means of sulphuric acid; some tri-calcium phosphate and nutritive salts are added and the solution is cooled to 86° F.

A barrel (50 liters) of this solution is taken, treated with 70 cc. of concentrated sulphuric acid and 5 grams of sodium hydrosulfate, and the pH is brought to between 2 and 3 by addition of more sulphuric acid. Fifty pounds of stock or commercial yeast are broken up and well mixed in this solution, which is allowed to stand for at least one hour. Thereupon the main 1500 gallon wort is aerated at the rate of 300 cu. ft. of air per minute and the yeast mixture is added. The temperature is kept between 82° and 91° F., and the pH is adjusted to between 4.0 and 4.8 by addition of calcium carbonate when necessary. At the end of 8 hours the Brix of the wort should drop from 6.5 to 0.7, provided that air supply, pH and temperature have been properly maintained, and if there is no infection. At the end, the percentage of invertase content of the yeast should have increased five or six times over that in the original yeast. The milk of yeast thus obtained is added at the rate of 0.07 per cent of yeast paste (60% moisture) on sucrose to the syrup from the evaporators, which should have a Brix of not more than 60 and a temperature not exceeding 140° F. After that, the syrup is well mixed with the invertase yeast and kept at the proper temperature in the inversion tanks for the length of time permitted by the factory economics. In general, the apparent purity will drop from 80 to 10 in 12 to 15 hours and the inversion will amount to 60 or 70

per cent. The inverting ability of the build-up yeast is somewhat less than that of commercial invertase yeast prepared by specialists, but there is a substantial saving in cost.

Standards Brands, Inc., holds patents for the production of special yeasts of high inverting power.

Invertase of quite satisfactory quality has been extracted in our laboratory and methods for its use will be described in a subsequent section of this paper. This invertase has recently been used in making high-quality table syrup at one of our sugar factories.

MANUFACTURE OF SYRUPS BY ACID INVERSION PROCESS

Formulas follow for making Table Syrup, Invert Syrup or Glucose, Hawaiian Sugar Honey, and Table and Cooking Molasses by the acid inversion method. Citric acid is used as the inverting agent in these formulas. The basic formulas for each are quite similar. The dissolved sugar material is heated to the boiling point, the source of heat removed and sufficient citric acid is added for the required amount of inversion which takes place during the period the material remains at high temperature. Sodium bicarbonate (baking soda) is then added to partially neutralize the acidity and the mixture boiled until the desired concentration is reached as evidenced by the specified final boiling temperature.

Each formula calls for an amount of sodium bicarbonate slightly in excess of the citric acid used, as we have found that it will be sufficient to remove the strong acid taste from the syrup but not enough to impair the taste from the use of too much soda. The pH before concentrating should be very close to 6.0.

Up to the point of partial neutralization with soda, almost any type of container except copper can be used. After adding the soda the acidity is so reduced that the boiling to final density can be made in copper containers if desired. It is preferable to concentrate the syrup under vacuum to avoid local overheating and possible impairment of the color and flavor due to too much caramelization. A formula for a table syrup which has a lower density than the other products with an end point of 108° C. (227° F.) will give a very palatable syrup if concentration is carried out either in the open or under vacuum.

All syrups if made in large batches should be run directly into the containers so that they will cool rather quickly. If finished syrup is to remain in a large kettle or tank for some length of time, provision should be made for its rapid cooling to protect the color and flavor, and when finally run into the containers the syrup should be reheated to about 175° F. to guard against mold growths.

Table Syrup:

The following proportions are those used for small-scale laboratory or home use, but they may be increased in the same proportion for large-scale use:

10 pounds raw sugar	10 pounds washed raw sugar
2 quarts water	2 quarts water
1½ ounces citric acid	¾ ounce citric acid
1¾ ounces sodium bicarbonate	¾ ounce sodium bicarbonate

Dissolve the sugar in the water and heat to boiling. Remove from heat and stir in the citric acid which has been dissolved in a small amount of water. Keep at 85° C. or above for one half to three quarters of an hour or until an apparent purity

of about 45 is obtained on a sample diluted to 15–20° Brix. Stir in the sodium bicarbonate slowly and then boil with constant stirring to 108° C. (227° F.). If a little heavier syrup is desired, increase the final temperature one or two degrees. As a guide to operations on a large scale, the sugar should be dissolved in water and diluted to a density of about 65° Brix. The citric acid should produce a reaction of about pH 3.0–3.2 for the inversion. After the inversion is completed the soda should give a reaction of about pH 6.0. If vacuum concentration is used the end point is about 8° above the boiling point of water at any given vacuum.

Hawaiian Sugar Honey:

A very palatable and light-colored product that closely resembles commercial honey can be made from washed raw sugar and citric acid.

10 pounds washed raw sugar
2 quarts water
 $\frac{3}{4}$ ounce citric acid
 $\frac{3}{8}$ ounce sodium bicarbonate

Dissolve the sugar in the water and heat to boiling. Remove from the heat and add the citric acid. Maintain the temperature at 85° C. (185° F.) or above for about 2½ hours or until the pol reading is negative in a sample diluted to 15–20° Brix. Then carefully add the sodium bicarbonate and evaporate under vacuum, to avoid caramelization and to protect the light color, until a boiling point elevation of 16° C. (29° F.) is reached. Break vacuum and run into containers. The Brix by refractometer is about 84°.

Table and Cooking Molasses:

This is a more highly flavored product than table syrup and is made from material representing the whole juice of the cane, in order to have it contain more of the flavor, color, mineral, organic and vitamin constituents than is obtainable when starting with raw, washed raw or refined sugar.

A convenient starting point for this product is after the juice has been clarified and is leaving the factory evaporators. This syrup is then partially inverted with citric acid to prevent crystallization at high density and then partially neutralized with sodium bicarbonate and finally evaporated to the desired density. The method of treatment would be as follows:

Heat the syrup to about 100° C. While stirring add one ounce of citric acid per gallon of syrup. Keep the temperature at about 85° C. or slightly above until a sample diluted to 15–20° Brix shows an apparent purity of 45–50. Stop the heat and while still stirring add 1¼ ounces of sodium bicarbonate per gallon of original syrup. It will probably be necessary to heat this syrup again to 100° C. and allow it to settle for from 2–6 hours to remove any sediment which may be formed. Most of the suspended material will settle out and the clear supernatant liquor can be decanted. The settlings can be filtered or returned to process via the liming tank and settlers. The clear syrup should be evaporated to the desired density, preferably under vacuum, with a boiling point elevation of 14° C. (25° F.). The density of the material made at the Station is about 84.3 refractometer Brix.

If the flavor of the product resulting from evaporator syrup is too strong, the batch may be made up before inversion using one-half evaporator syrup and one-

half raw sugar dissolved to the syrup density, or any other portions of raw or washed raw sugar may be used to obtain the desired flavor.

Glucose Syrup or Invert Syrup:

Glucose syrup is made by the inversion of all or nearly all of the sucrose. A longer time for more complete inversion of the sucrose to glucose is allowed than when making table syrups. Fairly complete inversion is accomplished by the following procedure:

10 pounds raw sugar
2 quarts water
1½ ounces citric acid
1¾ ounces sodium bicarbonate

Dissolve the sugar in the water and heat to boiling and add the citric acid. Keep at 85° C. (185° F.) or above for 5 hours or until a negative pol reading is obtained when a sample is diluted to 15–20° Brix. The closer the temperature is kept to 100° C. (212° F.) the more rapid the inversion will be. When a negative reading is obtained stir in the sodium bicarbonate slowly as the mixture will foam considerably. It is best to do the final boiling under vacuum with a final temperature elevation of 16° C. or 29° F., as this material will caramelize very easily at the higher densities.

CANDY FORMULAS

Plain Fudge:

(LARGE BATCH)
10 pounds raw or washed sugar
2 quarts water
⅛ ounce citric acid

(SMALL BATCH)
1½ pounds raw or washed sugar
¾ pint water (1½ cups)
½ gram citric acid (⅛ teaspoon or a little less than the amount you can heap on a dime)

Dissolve the citric acid in the water and add the sugar. Heat and boil until a thermometer indicates a final temperature of 115.5° C., or 240° F. Cool to 65° C., or 149° F., then stir or beat until change to dull appearance occurs as it starts "sugaring." Then quickly pour on a greased slab or pan. Cool and cut into squares. Flavoring may be added if desired after the final temperature has been reached. However, the original flavor of raw sugar changes during the boiling and the candy has a flavor resembling that of maple sugar.

Chocolate Fudge:

(LARGE BATCH)
10 pounds raw sugar
2 quarts water
⅛ ounce citric acid
1¼ pounds chocolate

(SMALL BATCH)
1½ pounds raw sugar
¾ pint water (1½ cups)
½ gram citric acid (⅛ level teaspoon)
¼ pound chocolate

Dissolve the citric acid in the water and add the sugar and chocolate. Heat slowly to boiling and boil to 114° C., or 237° F. Cool to 63° C., or 146° F., then stir until change to dull appearance occurs and pour on greased marble slab or pan. Cool and cut into squares. If flavoring is to be added, put in when boiling is completed. Nuts may be added just before pouring on slab. The amount of chocolate can be varied to suit individual taste.

Hard Candy:

(LARGE BATCH)
 10 pounds raw sugar
 1 gallon water
 ¼ ounce citric acid
 1 level teaspoon salt

(SMALL BATCH)
 1½ pounds raw sugar
 1 pint water (2 cups)
 1 gram citric acid (¼ level teaspoon)
 ¼ teaspoon salt

Dissolve the citric acid in the water and add the sugar and salt. Heat slowly to boiling and boil to 147° C. (297° F.). Nuts may now be added and the mixture poured on a greased marble slab or pan. Spread out thin with a spoon or spatula. Cool, break into pieces and store in airtight jars or moisture-proof containers.

Peanut Brittle:

(LARGE BATCH)
 10 pounds raw sugar
 1 gallon water
 ¼ ounce citric acid
 1 level teaspoon salt
 1½ ounces sodium bicarbonate
 6 pounds shelled, roasted or unroasted peanuts

(SMALL BATCH)
 1½ pounds raw sugar
 1 pint water (2 cups)
 1 gram citric acid (¼ level teaspoon)
 ¼ teaspoon salt
 1 level teaspoon sodium bicarbonate
 1 pound shelled, roasted or unroasted peanuts

Dissolve the citric acid in the water and add the sugar and salt. Heat to boiling and boil to 147° C. (297° F.), stirring frequently to prevent caramelization. Remove from heat source and add the sodium bicarbonate (baking soda) and stir quickly and thoroughly. Now add the peanuts, mix thoroughly and pour on a greased marble slab or pan. Spread out thin with a spoon or spatula. When cool, break into pieces and place in airtight or moisture-proof containers.

MANUFACTURE OF SYRUPS BY THE INVERTASE PROCESS

If mill juice or crusher juice is used, it must be clarified by any of several methods and then evaporated to a density suitable for the inversion or to about 37.5 to 40° Brix. Evaporator syrup can be used by either taking it off at a lighter than ordinary density or else it should be diluted with water or clarified juice to the desired density. Raw sugar, washed raw sugar or the washings from washed raw sugar may be used. If raw or washed raw sugar is used, it is dissolved and diluted to the proper density.

Several hours must be allowed for inversion. After sufficient inversion has taken place, the inverting action is stopped by heat or neutralization and the syrup is then evaporated to a heavy-bodied finished syrup. Under certain circumstances it may be necessary to filter the syrup before concentrating or to heat to 100° C. and allow to settle. The density of the solution, temperature, reaction and the amount and activity of the invertase determine the time required for the inversion and are the four important factors in the control of inversion in syrup making. The final sucrose purity desired is usually between 45 and 50.

Density for Inversion: Invertase acts rapidly upon sucrose in dilute solutions, but in concentrated liquors the rate of its reaction is greatly reduced. It becomes materially reduced above 40° Brix and for the most satisfactory work the density should be about 36° Brix when tested at 58–60° C.

Temperature for Inversion: The optimum temperature for inversion will vary slightly with different batches of invertase, but will usually be in the neighborhood of 55–60° C. (135° F.). Invertase is rapidly destroyed as the temperature is in-

creased above 60°, therefore the temperature should be held as near that point as possible but not above it. For practical operation the inverting tanks should be sufficiently insulated so that the temperature drop is not more than 10 degrees for the first 12 hours. The invertase should be added after the syrup has been heated to 60°. The syrup should be thoroughly mixed before and after adding the invertase. If it should be necessary to reheat during the course of inversion, it should never be with open steam coils and the syrup must be thoroughly stirred to prevent any local overheating. However, if invertase is added to a large volume of syrup in a well-insulated tank, the cooling will ordinarily be so slow that, for all practical purposes, the temperature will remain high enough during the time necessary for the required amount of inverting action to take place.

Reaction (pH): Invertase exerts its greatest activity in a slightly acid solution. The optimum reaction is usually at about pH 4.8 to 5.2. If the syrup is made by heating and skimming raw or crusher juice without the addition of lime, the natural acidity will be just about right for optimum inversion and no adjustment will be necessary. If clarified juice, syrup, or sugar is used the reaction must be adjusted to between pH 4.8 and 5.2. Any pure acid can be used for this purpose. Citric acid, being produced locally, can be used since the required reaction is not low enough and the temperature not high enough to cause damage to the equipment.

Time for Inversion and Amount of Invertase: The time for inversion to take place and the amount of invertase required are dependent one upon the other, together with the factors already mentioned. Other factors remaining constant, the inversion will be faster with larger quantities of invertase. Conversely, the amount of invertase can be reduced if sufficient time is available for a slower inversion. Capacities are usually calculated to allow from 12 to 16 hours for inversion.

If the activity of any particular batch of invertase is not known it should be determined under controlled temperature conditions. The following example will illustrate the activity of a batch of invertase prepared at the Station:

Washed raw sugar syrup having a purity of 99.0 and a density of 38.6° refractometer solids was used for this test. The activity was determined at varying reactions and at different temperatures, *i.e.*, reactions of pH 4.6, 5.0 and 5.4 and temperatures of 52, 58 and 64° C. Invertase was added at the rate of 1 ml. per pound of sugar in solution. The average drop in purity per hour at 58° C. was 5.6 at pH 4.6, 5.5 at pH 5.0, and 5.0 at pH 5.4. This indicated the optimum reaction to be between pH 4.6 and 5.0. The average purity drop per hour for different temperatures was 4.4 at 52°, 5.3 at 58° and 5.8 at 64°. While the indicated rate was higher at 64°, the temperature was actually too high, for the bihourly figures showed that the invertase was being destroyed. The drop was 6.75 for the first two-hour period, 5.8 for the second two-hour period and 5.0 for the third two-hour period. The figures actually showed that the optimum activity was obtained at a reaction of pH 5.0 and a temperature of 60° C. Under these conditions the average purity drop per hour was 6.0+.

From the above data the requirements for a 500-gallon tank of syrup can be estimated as follows: 500 gallons of syrup made from washed raw sugar at 38.5° Brix and 98.5 purity would contain approximately 1840 pounds of sucrose or sugar in solution. If it is required that this be inverted from 98.5 purity to 48.5 purity the total purity drop is 50 points. Under the conditions of the above test, 1 ml. of invertase per pound of sugar in solution at pH 5.0 and 60° C. would produce a

purity drop averaging about 6.0 points per hour. Therefore, if invertase were added in the same proportions and the temperature maintained at 60° C., 8½ hours would be required. If 17 hours could be allowed for the inversion, the invertase requirement could be reduced to one half the quantity. The usual practice in syrup plants is to start the inversion about 4:00 o'clock in the afternoon and allow it to proceed overnight for a total of about 16 hours. Allowing for a temperature drop of from 8 to 10 degrees, the amount of invertase could be reduced by about one quarter or from 1840 ml. to about 1400 ml. and the amount of inversion should still be sufficient. Inversion will be still more rapid if the density of the syrup is reduced to about 35° Brix, but at the same time this means more water to be evaporated.

Directions for Inversion with Invertase: Evaporate clarified cane juice rapidly to about 35° Brix at the evaporator temperature or dilute finished syrup, washed or unwashed raw sugar, to the same density and run it into the inversion tank. Adjust the reaction to pH 5.0 and the temperature to 60° C. (140° F.). Add the required amount of invertase to produce the desired degree of inversion in the allotted time. Then mix thoroughly with air or by mechanical means. If the insulation is sufficient, the solution may be allowed to stand for the necessary time without further attention. If it is necessary to reheat during inversion it should not be done with an open steam coil and great care should be exercised to prevent any local overheating around the heating units.

These instructions should be followed as closely as possible, but slight variations will not greatly affect the results. For instance a variation of two or three degrees in Brix is permissible and a similar difference in temperature is allowable. Also the reaction range may vary from 4.6 to 5.2 pH without affecting the results to a material extent.

The course of the inversion should normally be followed by "purity" or "pol" determinations about every three hours. This is not necessary except to determine the degree of inversion near the end of the cycle. However, such a routine if it can be followed will indicate whether inversion is proceeding as it should and if not, allow the operator to correct the difficulty before too much time has been lost. If the above conditions are maintained it should be possible to reproduce the results from day to day with the same batch of invertase. It may be necessary to change the temperature or the reaction slightly for different batches of invertase.

Inversion is stopped either by heating to 75°–80° C. or by neutralization. Lime is the most economical neutralizing agent. Neutralization is optional; the unneutralized syrup has a slightly sharper taste which some people prefer. Neutralization tends to equalize the intensity of sweetness and flavor and produce a somewhat smoother product.

Filtration: It will usually be found necessary to filter the syrup after inversion or to heat it and allow it to settle to secure a clear syrup. It may also be necessary to filter after final evaporation. The addition of a very small amount of phosphate in the form of Ammo-phos followed by lime to a reaction of about pH 7.6 effects a marked clarification and does not give any difficulty in the filtration after inversion has been completed and the syrup heated to 75–80° C. Filtration is preferred to settling if the equipment is available. A second filtration is sometimes practiced after evaporation to final density. If filter aid is available it will materially assist filtration.

A secondary precipitation has occurred in some instances after the final syrup has stood for some time. The cause of this has not been definitely determined, but we have been informed from one source that this has been overcome by heating the heavy syrup to not over 75° C.

Evaporation to Final Density: Evaporation may be accomplished under vacuum or by "open-kettle" boiling at atmospheric pressure. The choice at present would depend largely upon the equipment available. Evaporation under vacuum is more readily controlled and is conducive to a more uniform product. The two methods produce syrup with some differences in color and flavor; the open-kettle method is usually accompanied by some caramelization, is slightly darker in color and has a slightly sharper flavor.

Density: The standard commercial density is about 72° Brix. Some prefer a slightly lower or a slightly higher density. Samples of syrup produced locally range from 69 to 75° Brix. Syrup does not readily ferment in warm weather at 78–80° Brix, but proper canning or bottling is depended upon to prevent fermentation at densities around 70–72° Brix. Practically no fermentation has been observed in samples of locally produced table syrup.

Purity: Reduction in apparent purity to between 50–55 is sufficient to prevent crystallization at a density of approximately 72° Brix, but if it is desired to carry the density at 78–80° Brix the purity should be reduced to about 45.

Cooling Syrup: Syrup should not be placed in storage tanks while hot, but should be cooled somewhat during its passage from the evaporators to the storage tanks. The temperature should be reduced to at least 180° F., or about 82° C., rather rapidly after evaporation if it has been concentrated by the open-kettle method to prevent any change in color or flavor.

Canning Syrup: The canning of table syrup is, fortunately, not a difficult operation, but the subject is worthy of more discussion than can be devoted to it here. It is fully covered in U.S.D.A. Bulletin 1370, 1925, p. 58, in a section by W. L. Owen, from which the following is taken:

Cane sirup may be preserved in cans [or bottles] with little chance of failure, if certain simple rules are observed. There are no great difficulties to be overcome, and there is no reason why, even with the simplest equipment, cane sirup can not be canned with minimum loss from spoilage. The three general conditions necessary are (1) to fill the can with sirup at the proper temperature [170° to 180° F.], (2) to obtain airtight closure of the can, and (3) to avoid long retention of heat by the sirup both before and after canning.

Glucose or Invert Syrup Made with Invertase: Complete inversion of all of the sucrose, to form glucose or invert sugar in the production of glucose or invert syrup, can be accomplished by the use of invertase in a manner similar to that just described. In order to produce a high quality of invert syrup it would be necessary to start from washed raw sugar or even from refined sugar. Other steps in the process would not be greatly changed, except that the inversion would be carried to a minus polariscope reading of about 17°.

* * * * *

U.S.D.A. Bulletin No. 1370, 1925, contains a compilation of valuable information on the subject "Sugar-Cane Sirup Manufacture" and can be procured from The Superintendent of Documents, Government Printing Office, Washington, D. C., for 10 cents per copy.

PREPARATION OF INVERTASE FOR SYRUP MAKING

Invertase with reasonable inverting power (activity) may be prepared from ordinary compressed yeast as follows:

Method in Brief: Break up five pounds of compressed yeast into a wide-mouthed container. Add 30 ml. of chloroform, cover with a cloth and allow to stand for 36 to 48 hours at room temperature. This mass is then filtered and the filtrate stored in a refrigerator. If a refrigerator is not available, toluene may be poured on top to form a layer of about $\frac{1}{8}$ inch.

Details of Method: The following details have been learned from our experience in the Station laboratory and may be of some value.

Invertase with quite satisfactory activity has been prepared from compressed yeast produced in the Station's pilot plant.

The chloroform should be quite well distributed over the surface of the yeast. It may even be mixed into the yeast with a stirring rod or stick although this is not necessary.

After a varying length of time the mass begins to liquify and become fluid. This time varies from an hour and a half up to five or six hours and seems to depend upon the age of the yeast and the temperature. At this stage the entire mass should be gently stirred and mixed to break up lumps and form a uniform mixture. Also at this stage the mass may foam or froth considerably and if not watched or if in too small a container a large portion may be lost. Foaming over can be prevented by gently stirring down the foam occasionally. Too vigorous stirring of the entire mass may cause more foaming.

When the first vigorous frothing has ceased, add about 200 ml. of water and gently mix into the mass and allow it to stand. There is usually then no further danger of foaming over.

Use a container of ample size to allow for from three to five times the volume occupied by the crumbled yeast. A wide container with wide mouth is preferable. An earthenware crock is suitable. At the Station a four-gallon enameled ware cooking pot with a ratio of 1:1 or 1:3 diameter to height is used for five-pound lots of yeast, a six-gallon container for ten-pound lots and a ten-gallon container for 20-pound lots.

The procedure at the Station is to mix the yeast and chloroform about noon, then the dangerous stage of frothing is over by closing time and the mass is allowed to stand until the second morning following when filtration is started.

A number of methods have been tried in attempting to effect a rapid separation of the liquid from the mass of yeast cells, for this filtration is very slow. Attempts with suction, pressure or centrifuge have not been satisfactory. The following procedure is now used: Six to a dozen flasks with 6-inch funnels are fitted with 8- to 12-inch fluted filter papers. A cheap grade of coarse filter paper is permissible. These are filled and allowed to drain most of the day. The material remaining on the filter papers is poured back into the original container, the funnels fitted with new papers are again filled and these are allowed to drain overnight. The same procedure is followed the next morning if necessary, or, until the entire mass is filtered. The heavy mass remaining on and clinging to the filter paper with the paper itself is accumulated in a separate container. To this is added a volume of water equal to the volume of filtrate which was extracted and about 10 ml. of chloroform. The mass of yeast and filter paper is broken up and stirred into a homo-

geneous mass, allowed to stand overnight and filtered in the same manner as the original material. This filtrate is mixed with the main filtrate and placed in the refrigerator.

The filtrate, of course, contains the invertase. It may be further purified and concentrated, but this is a long tedious process and appears unnecessary for present purposes. The activity (inverting power) of this crude invertase is not as high as the commercial product purchased on the market, but it serves as a very good substitute under present conditions. The optimum temperature and reaction may vary somewhat in different batches. Therefore, it seems preferable to prepare fairly large batches and then determine the optimum temperature in the laboratory and apply these findings in the plant.

An example of the activity of a batch of about 20 liters of crude invertase prepared in this laboratory follows:

Washed raw sugar syrup at 99° refractometer purity and 38.6° refractometer solids was used in the test. Invertase was added in the proportion of 1 ml. per pound of sugar.

Purity drop	Different reactions at a temperature of 58° C.			Different temperatures at a reaction of pH 5.0		
	4.6	5.0	5.4	52	58	64
2 hours	11.6	11.2	11.3	9.1	11.4	13.5
4 hours	22.4	22.4	21.6	17.6	22.4	25.2
6 hours	32.6	32.6	30.1	25.5	32.1	35.2
Average drop per hour...	5.5	5.5	5.0	4.3	5.4	5.8

These figures show little difference in the activity at reactions of pH 4.6 or 5.0, but do indicate a slight dropping off at pH 5.4. The figures show a decided increase in activity with increased temperature from 52° to 64° for the first two-hour period, but the increase is not sustained at 64° for the 4- and 6-hour periods, and indicate that 64° C. is somewhat too high for this invertase. The optimum conditions for this batch would then be a reaction of pH 5.0-5.2, (maintaining the higher reaction to conserve acid and reduce corrosion of equipment) with a temperature of 60° C. These conditions should then give a purity drop of about 6 points per hour. With these data the necessary amount of invertase to invert a given amount of sugar in a given time could be estimated, or conversely, the time interval required for a given amount of sugar and a given amount of invertase could be calculated.

Proper temperature control is the most important factor in obtaining the maximum activity in inversion with invertase. The invertase should be added to the syrup *after* the syrup has been brought to the desired temperature, and if it is necessary to apply heat during the course of the inversion it should not be from open steam coils and care should be taken to insure thorough mixing to prevent local overheating.

The subject of invertase preparation has not been given a great deal of study at the Station and, doubtless, the method and technique herein described can be considerably improved. However, invertase prepared as above has been used to produce a very satisfactory syrup and definite progress is being made toward filtration of the treated yeast in a filter press.

References on Invertase Preparation:

- Handbook of Sugar Analysis—Browne, C. A., 1st Edition, pp. 669-670, 1912.
 Sugar Analysis—Browne, C. A., & Zerban, F. W., 3rd Edition, pp. 428-437, 1941.

The Synthesis of Sucrose in the Sugar Cane Plant*—I

By CONSTANCE E. HARTT

In providing for its own nourishment and the growth of its own body, the noble sugar cane plant is wont to carry its sugar in transit and in storage in the superior form of sucrose. In recent years, however, the sugar canes cultivated in Hawaii do not seem to be maintaining as high a standard in this respect as formerly but are inclined to carry more and more of their sugar in the form of glucose and its counterpart fructose. As a result of this delinquency, the trend in the quality of our sugar cane juices is downward and we are unable to recover as much sucrose therefrom as we desire. Since we have proved that the formation of glucose precedes the formation of sucrose in the sugar cane plant, studies to determine the factors influencing the conversion of glucose to sucrose may point the way to means of improving the quality of our sugar cane juices.

The transformations of sugars in the leaves of plants have been studied by several investigators. Russian physiologists have been particularly active, and many of their results have recently been summarized (49)†. Other investigators active in this field include Virtanen and Nordlund (83), Nurmia (née Nordlund) (65), Leonard (51), and Hassid (36, 58). No attempt will be made to review their results in this report, but their works are cited for the benefit of anyone who might wish to learn the scope of the investigations being carried on elsewhere in this particular field of research.

Our studies of the synthesis of sucrose by excised blades of sugar cane have been carried on over a period of more than five years and two reports (33, 35) have already been published. Our investigations are not yet completed, but it seems advisable at this time to record our progress to date in permanent form. For the sake of convenience, this report is divided into four parts. The first deals with sugar transformations in separate organs, entire stalks, and entire plants; also with time, temperature, chlorophyll, aeration and mutilation. The second part deals with the effects of several inorganic and organic compounds. The third part deals with specific inhibitors. The fourth part deals with the interrelationships of the factors treated in the first three parts and an attempt is made to explain the sequence of events in the synthesis of sucrose.

The results lead to the conclusion that the formation of sucrose fits into the general scheme of carbohydrate metabolism already established by the studies of Cori (10-12), Hanes (29), and others. Because inhibiting the formation of fructose diphosphate inhibits the formation of sucrose, whereas inhibiting the breakdown of fructose diphosphate increases synthesis, we are led to conclude that fructose diphosphate is a stepping stone in the formation of sucrose by the sugar cane plant.

* Presented in part as a presidential address to the Hawaiian Botanical Society, December 1, 1941, Honolulu, Hawaii.

† Numbers in parentheses refer to literature citations at the end of the fourth part of this paper.

The Hawaiian sugar cane varieties 109 and 32-8560 were used in this investigation. Tests showed that these varieties are equally suitable for these studies. In the experiments using blades, the plants were grown in the field at the Experiment Station and had received optimum fertilization and irrigation. In the experiments using roots, the plants were grown in complete nutrient solution and with aeration in tanks of approximately 25 gallons capacity. Blades were cut from the plants in the morning, and were always taken from the same position on the plant. Generally eight blades were used in each series. Roots were shaved from stems, washed, dried in a centrifuge, and weighed—these procedures being conducted uniformly for each experiment.

Samples of blades and roots were ground at once and preserved for analysis, these samples being designated "initial controls." The other series were placed in their respective solutions, in a constant temperature room in absolute darkness. After the experimental period, generally 24 hours, they were washed, dried, ground, and sampled.

The methods of sampling and analysis were the same as those previously described (35).

All of the results of the moisture determinations are expressed as percentages on the wet-weight basis; all of the results of the sugar determinations are expressed as percentages on the dry-weight basis unless otherwise specified.

PART I

THE INTERCONVERSION OF GLUCOSE AND FRUCTOSE AND THE FORMATION OF SUCROSE IN DETACHED ORGANS OF THE SUGAR CANE PLANT

1. The formation of sucrose from glucose:

Many tests have been conducted in which blades detached from the plant have been placed with their cut ends in five per cent solutions of glucose. The blades absorb some of the glucose and increase in percentage of sucrose. The results of a typical test are recorded in Table I which shows that blades supplied with glucose gained considerably more sucrose than reducing sugars. This gain in sucrose is considered to be an actual synthesis of sucrose from the glucose supplied, rather

TABLE I
MOISTURE AND SUGAR PERCENTAGES OF BLADES SUPPLIED WITH 5%
GLUCOSE FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	68.07 \pm 0.076	0.817 \pm 0.027	2.905 \pm 0.014	3.875 \pm 0.012
5% glucose	63.61 \pm 0.024	1.820 \pm 0.029	6.452 \pm 0.006	8.612 \pm 0.036

than a rearrangement of the carbohydrates already within the blades, because similar blades supplied with an inorganic solution of the same osmotic concentration as the glucose showed no increase in sucrose (33).

The formation of sucrose from glucose can also take place in sheaths, as shown in Table II. The blades and sheaths mentioned in Tables I and II were taken from the same stalks at the same time, and the results may therefore be compared. A convenient method of comparison is afforded by the synthetic efficiency, which is the percentage of glucose absorbed that is converted into sucrose. The synthetic effi-

TABLE II
MOISTURE AND SUGAR PERCENTAGES OF SHEATHS SUPPLIED
WITH 5% GLUCOSE FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	75.56 ± 0.195	3.715 ± 0.001	7.777 ± 0.019	11.900 ± 0.002
5% glucose	74.62 ± 0.257	3.986 ± 0.002	9.870 ± 0.067	14.380 ± 0.071

ciencies of these blades and sheaths, calculated from Tables I and II, are found in Table III. Although the sheaths gained less total sugar and sucrose than the blades, the process of synthesis operated fully as well, in fact better, in the sheaths than in the blades.

TABLE III
THE SYNTHETIC EFFICIENCY OF BLADES AND SHEATHS,
CALCULATED FROM TABLES I AND II

Series	Gain in total sugar %	Gain in sucrose %	Synthetic efficiency
Blades	4.737	3.547	74.87
Sheaths	2.480	2.100	84.67

The formation of sucrose from glucose was also studied in the stem. The upper portion of the stem was used, from which all leaves were removed. A wild variety of sugar cane, *Sacharum robustum* (Molokai 1293) from New Guinea, was used in these tests, because the cultivated or noble canes already contain so much sugar that they cannot absorb any more. The results are presented in Table IV. The synthetic efficiency of the stems was 45.31. It is evident that sucrose can be made

TABLE IV
MOISTURE AND SUGAR PERCENTAGES IN STEMS SUPPLIED
WITH 10% GLUCOSE FOR 48 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	82.16 ± 0.410	5.139 ± 0.256	5.23 ± 0.067	10.64 ± 0.181
10% glucose	83.78 ± 0.105	6.810 ± 0.032	6.68 ± 0.048	13.84 ± 0.029

from glucose in the stem of sugar cane, but that this process is not as efficient in the stem as in the blade and sheath.

The results of studies with entire stalks are presented in Table V. Stalks of variety *Saccharum robustum* (Molokai 1293), age 1½ years, were cut at the joint

TABLE V
MOISTURE AND SUGAR PERCENTAGES IN ENTIRE STALKS OF *SACCHARUM*
ROBUSTUM, SUPPLIED WITH 10% GLUCOSE FOR 48 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Blades:				
Initial control	66.57 ± 0.091	0.571 ± 0.018	1.175 ± 0.019	1.808 ± 0.039
10% glucose	65.40 ± 0.835	1.089 ± 0.050	3.096 ± 0.187	4.349 ± 0.247
Sheaths:				
Initial control	77.46 ± 0.162	1.925 ± 0.251	1.803 ± 0.091	3.824 ± 0.347
10% glucose	75.32 ± 0.386	2.779 ± 0.266	5.368 ± 0.021	8.430 ± 0.288
Stems:				
Initial control	82.16 ± 0.410	5.139 ± 0.256	5.23 ± 0.067	10.64 ± 0.181
10% glucose	76.73 ± 0.696	5.531 ± 0.408	12.58 ± 0.720	18.78 ± 0.353

at which the lowest living green leaf was attached, and were placed in 10 per cent solutions of glucose. There were three stalks per series, and each series was run in duplicate. The results in Table V are the averages of these duplicate series. After 48 hours the blades, sheaths, and stems were separated and sampled. The percentage of sucrose more than doubled in all three organs. The question arises, as to whether the increase in sucrose in the stem is due to synthesis *in situ* or to translocation from the leaves. This question may be answered by reference to Table IV, in which excised stems of the same plants as those in Table V were used. The results with excised stems show that sucrose can be synthesized from glucose in the stem, but the amount made in that way by no means accounted for all of the increase in sucrose in the stems of the entire stalks. Therefore both synthesis *in situ* and translocation from the leaf are involved in the storage of sucrose in the stem.

The absorption of glucose and formation of sucrose were next studied in entire plants. For this purpose we used plants of 2-4 months of age, which had been grown in complete nutrient solution. The plants were placed with their roots in five per cent solutions of glucose for 48 hours, with fresh glucose supplied after 24 hours. The results of a typical test with three plants per series are recorded in Table VI, which shows that the roots absorbed glucose and made sucrose. In the

TABLE VI
MOISTURE AND SUGAR PERCENTAGES IN ENTIRE PLANTS
SUPPLIED WITH 5% GLUCOSE FOR 48 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Blades:				
Initial control	73.81 \pm 0.100	2.227 \pm 0.007	4.195 \pm 0.000	6.643 \pm 0.006
5% glucose	73.80 \pm 0.100	3.792 \pm 0.003	2.390 \pm 0.001	6.308 \pm 0.001
Sheaths:				
Initial control.....	82.37 \pm 0.038	7.154 \pm 0.006	8.867 \pm 0.057	16.488 \pm 0.065
5% glucose	79.81 \pm 0.029	10.151 \pm 0.013	5.762 \pm 0.017	16.217 \pm 0.031
Stems:				
Initial control	84.47 \pm 0.081	9.863 \pm 0.048	21.113 \pm 0.009	32.087 \pm 0.058
5% glucose	82.96 \pm 0.038	9.795 \pm 0.030	22.804 \pm 0.056	33.799 \pm 0.089
Roots:				
Initial control	88.99 \pm 0.024	4.427 \pm 0.120	6.439 \pm 0.086	11.204 \pm 0.211
5% glucose	88.50 \pm 0.091	8.097 \pm 0.016	10.639 \pm 0.048	19.296 \pm 0.035

blades and sheaths there were no gains in sucrose, and in the stems the gain in sucrose was much smaller than in the roots. The failure of the tops to gain in sucrose was typical of these experiments with entire plants, whereas when stalks were used, considerable increases in sucrose occurred in blades, sheaths, and stems, as shown in Table V. It would seem that translocation of sugar from roots to stems was hindered in some way. Since it is well known that roots ordinarily obtain their supply of sugar from the tops, these results suggest the existence of polarity in translocation of sugar from tops to roots.

The data recorded in Table VI may be taken to indicate that the synthesis of sucrose from glucose can take place in roots. To study this question further, an experiment was conducted in which both attached and detached roots were used. The results are presented in Table VII. Since the attached roots made considerably more sucrose than the detached roots, it was concluded that some substance essential for synthesis was supplied from the tops to the roots (34). Studies of the effects

of aeration, of vitamins, and of hormones were therefore undertaken, and are described elsewhere in this report.

TABLE VII

MOISTURE AND SUGAR PERCENTAGES IN ATTACHED AND DETACHED
ROOTS SUPPLIED WITH 5% GLUCOSE FOR 48 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	90.27 \pm 0.219	0.275 \pm 0.029	2.539 \pm 0.053	2.948 \pm 0.085
Attached roots	90.25 \pm 0.138	3.291 \pm 0.060	4.214 \pm 0.088	7.728 \pm 0.032
Detached roots	90.89 \pm 0.052	7.113 \pm 0.019	2.803 \pm 0.037	10.064 \pm 0.020

The experiments already described lead to the conclusion that the formation of sucrose from glucose can take place in blades, sheaths, stems, and roots of the sugar cane plant, when supplied with glucose. This process takes place in absolute darkness. To compare this process with the formation of sucrose in photosynthesis, detached blades were placed in water in sunlight for 11½ hours, and similar blades were supplied with 10 per cent glucose in the dark for the same length of time. For this test, blades were taken from different levels on the plant. Counting the leaf with the highest visible ligule as leaf number 1, our routine method is to use leaves 1 and 2 in these studies. For this particular test, we compared leaves 1 and 2 with older leaves, numbers 7 and 8. The results are recorded in Table VIII. Blades 7 and 8 increased in sucrose more than blades 1 and 2, whether by photosynthesis or by synthesis in the dark. The difference was about the same: by photosynthesis, 0.493; and by synthesis in the dark, 0.644. This is in agreement with the theory that the synthesis of sucrose takes place by the same mechanism whether the glucose is supplied artificially as in these tests or naturally by the process of photosynthesis. Further evidence that we are dealing with the natural process of sucrose formation is afforded by other experiments in this report.

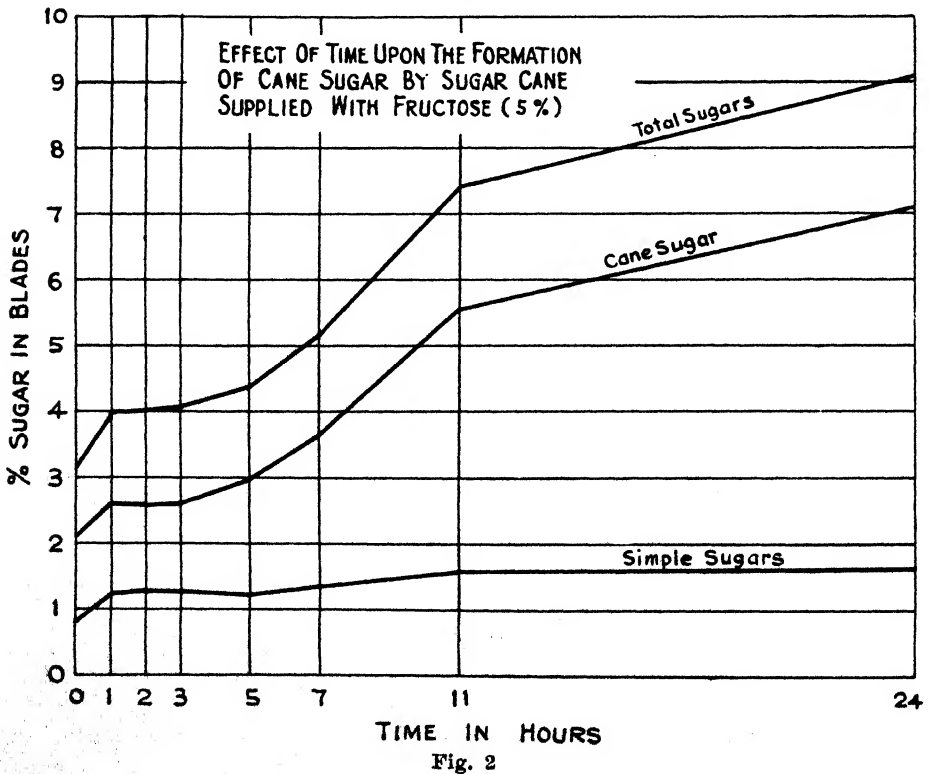
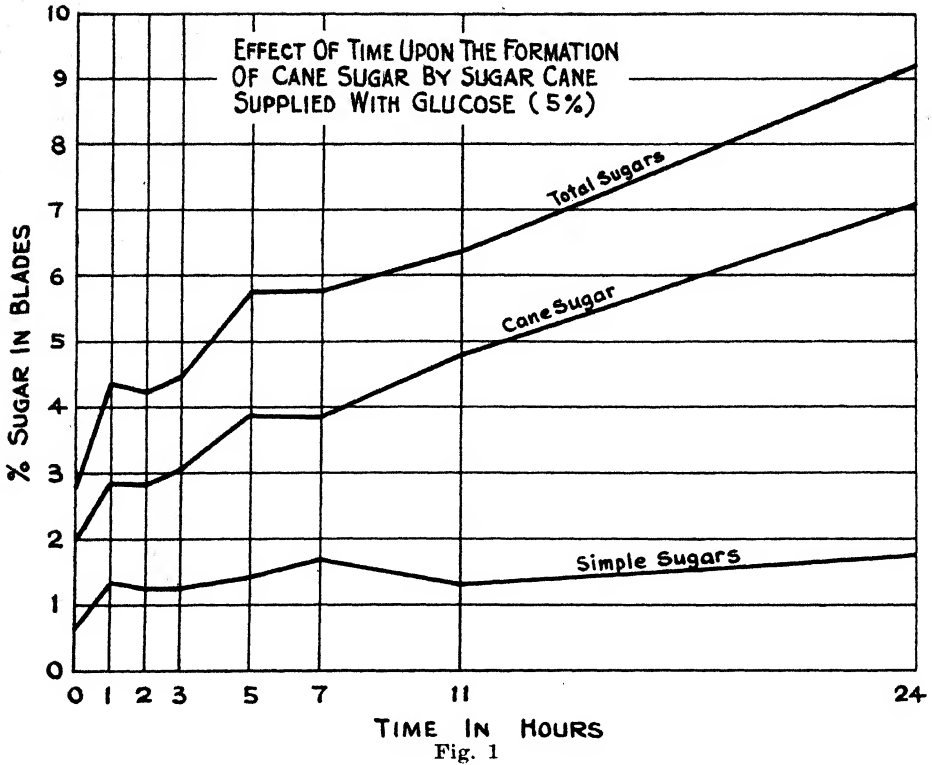
TABLE VIII

MOISTURE AND SUGAR PERCENTAGES IN DETACHED BLADES IN WATER
IN SUNLIGHT, AND IN BLADES SUPPLIED WITH 10%
GLUCOSE IN THE DARK, FOR 11½ HOURS

Series	Moisture	Reducing sugars	Sucrose	Gain in sucrose	Total sugars
Initial controls:					
Blades 1 & 2.....	73.01 \pm 0.057	1.621 \pm 0.010	3.913 \pm 0.036		5.740 \pm 0.028
Blades 7 & 8.....	72.60 \pm 0.062	1.467 \pm 0.055	3.942 \pm 0.027		5.617 \pm 0.026
Water in sunlight:					
Blades 1 & 2.....	69.48 \pm 0.062	2.229 \pm 0.012	6.545 \pm 0.003	2.632	9.118 \pm 0.016
Blades 7 & 8.....	69.58 \pm 0.281	2.233 \pm 0.028	7.067 \pm 0.013	3.125	9.673 \pm 0.014
Glucose in darkness:					
Blades 1 & 2.....	70.89 \pm 0.033	2.853 \pm 0.001	6.154 \pm 0.012	2.241	9.331 \pm 0.014
Blades 7 & 8.....	70.01 \pm 0.024	3.829 \pm 0.039	6.827 \pm 0.024	2.885	11.016 \pm 0.064

2. Time and the formation of sucrose:

Detailed studies of time and the formation of sucrose from glucose or fructose have already been published (35). The hourly formation of sucrose in blades supplied with glucose is depicted in Fig. 1, and in blades supplied with fructose in Fig. 2. The percentage of cane sugar increased considerably more than the percentage of simple sugars, although the blades were supplied with simple sugars. These



curves are very similar to curves obtained in studies of fluctuations of sugars in attached blades in the open during the day and the night (32).

The daily formation of sucrose in sugar cane blades supplied with glucose is shown graphically in Fig. 3. The percentage of sucrose increased for nearly two weeks, yet the percentages of glucose and fructose showed only minor fluctuations.

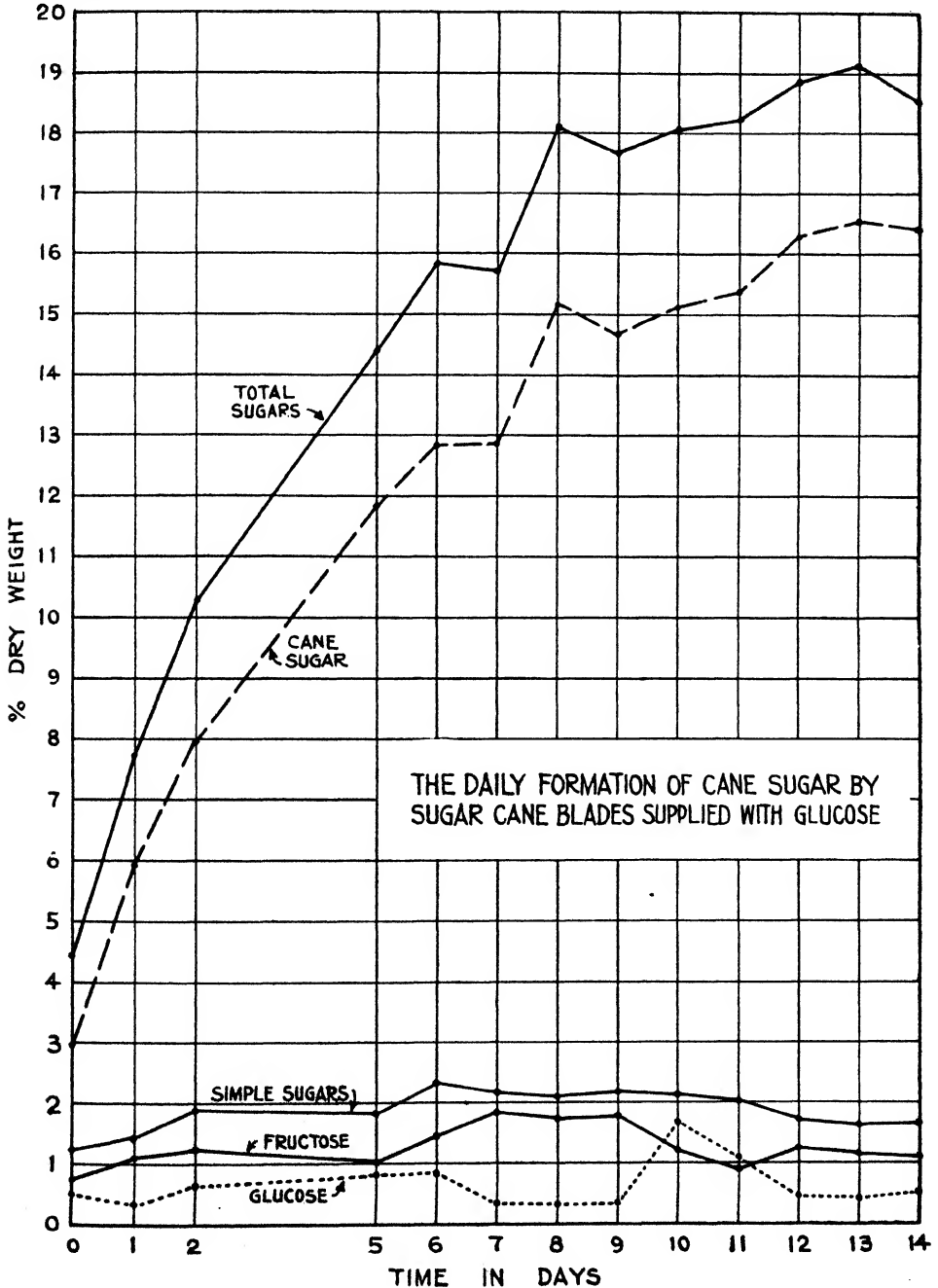


Fig. 3

The time of day when the leaves are taken from the plant is another factor affecting synthesis. In all routine tests the leaves are taken between eight and nine o'clock in the morning. To determine whether there is a diurnal fluctuation in the mechanism of synthesis, blades were taken one day at 9 a.m. and 9 p.m., and another day at 4:30 a.m. and 4:30 p.m. All the blades remained in 5 per cent glucose for exactly 24 hours. The results are presented in Table IX. The gain in total sugar and sucrose and the synthetic efficiency, calculated from Table IX, are shown in Table X. The formation of sucrose from glucose, fructose, or both glucose and fructose was least efficient in blades taken at 9 p.m. This was not due to lack of light, because blades taken at 4:30 a.m. were as efficient as blades taken in the daylight hours.

TABLE IX

MOISTURE AND SUGAR PERCENTAGES IN BLADES TAKEN AT DIFFERENT TIMES OF THE DAY AND SUPPLIED WITH 5% GLUCOSE OR FRUCTOSE FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control:				
9:00 a.m.	72.38 \pm 0.143	0.929 \pm 0.010	2.902 \pm 0.016	3.983 \pm 0.006
4:30 p.m.	70.58 \pm 0.110	1.098 \pm 0.017	5.145 \pm 0.027	6.514 \pm 0.045
9:00 p.m.	72.05 \pm 0.038	1.017 \pm 0.010	4.034 \pm 0.027	5.263 \pm 0.018
4:30 a.m.	72.09 \pm 0.057	1.041 \pm 0.012	2.704 \pm 0.013	3.888 \pm 0.027
Supplied with glucose:				
9:00 a.m.	70.85 \pm 0.038	1.525 \pm 0.001	6.091 \pm 0.001	7.937 \pm 0.003
4:30 p.m.	69.69 \pm 0.019	1.395 \pm 0.013	7.258 \pm 0.008	9.035 \pm 0.005
9:00 p.m.	72.13 \pm 0.119	1.650 \pm 0.003	5.777 \pm 0.001	7.731 \pm 0.005
4:30 a.m.	69.92 \pm 0.086	1.359 \pm 0.001	5.000 \pm 0.002	6.622 \pm 0.003
Supplied with fructose:				
9:00 a.m.	71.08 \pm 0.110	1.296 \pm 0.008	4.942 \pm 0.024	6.499 \pm 0.017
4:30 p.m.	69.12 \pm 0.024	1.500 \pm 0.000	7.410 \pm 0.008	9.300 \pm 0.008
9:00 p.m.	71.58 \pm 0.086	1.473 \pm 0.006	6.021 \pm 0.011	7.811 \pm 0.006
4:30 a.m.	70.39 \pm 0.043	1.283 \pm 0.026	5.238 \pm 0.018	6.798 \pm 0.045
Supplied with glucose and fructose:				
9:00 a.m.	69.84 \pm 0.091	1.138 \pm 0.002	5.677 \pm 0.011	7.114 \pm 0.014
4:30 p.m.	68.19 \pm 0.024	1.386 \pm 0.010	7.598 \pm 0.021	9.384 \pm 0.033
9:00 p.m.	71.33 \pm 0.000	1.320 \pm 0.006	6.025 \pm 0.004	7.663 \pm 0.010
4:30 a.m.	70.51 \pm 0.000	1.255 \pm 0.003	4.799 \pm 0.012	6.307 \pm 0.010

TABLE X

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY IN BLADES TAKEN AT DIFFERENT TIMES OF THE DAY AND SUPPLIED WITH 5% GLUCOSE OR FRUCTOSE FOR 24 HOURS, CALCULATED FROM TABLE IX

Series	Gain in total sugar %	Gain in sucrose %	Synthetic efficiency
Supplied with glucose:			
9:00 a.m.	3.954	3.189	80.65
4:30 p.m.	2.521	2.113	83.81
9:00 p.m.	2.468	1.743	70.62
4:30 a.m.	2.734	2.296	83.97
Supplied with fructose:			
9:00 a.m.	2.516	2.040	81.08
4:30 p.m.	2.786	2.265	81.29
9:00 p.m.	2.548	1.987	77.98
4:30 a.m.	2.910	2.534	87.07

Supplied with glucose + fructose:

9:00 a.m.	3.131	2.775	88.62
4:30 p.m.	2.870	2.453	85.47
9:00 p.m.	2.400	1.991	82.95
4:30 a.m.	2.419	2.095	86.60

The percentages of glucose and fructose are recorded in Table XI which shows that the series given glucose at 9 a.m. made fructose, but the series given glucose at 9 p.m. accumulated glucose and lost fructose. It would seem that the blades taken

TABLE XI
FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES TAKEN AT DIFFERENT
TIMES OF THE DAY AND SUPPLIED WITH 5% GLUCOSE
OR FRUCTOSE FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control:				
9 a.m.	0.764 ± 0.005		0.165 ± 0.005	
9 p.m.	0.652 ± 0.022		0.365 ± 0.012	
Supplied with glucose:				
9 a.m.	1.004 ± 0.066	0.240	0.521 ± 0.065	0.356
9 p.m.	0.484 ± 0.025	-0.168	1.166 ± 0.021	0.807
Supplied with fructose:				
9 a.m.	0.653 ± 0.025	-0.111	0.643 ± 0.034	0.478
9 p.m.	0.649 ± 0.001	-0.003	0.823 ± 0.007	0.458
Supplied with glucose + fructose:				
9 a.m.	0.582 ± 0.048	-0.182	0.556 ± 0.051	0.391
9 p.m.	0.998 ± 0.004	0.346	0.325 ± 0.004	-0.040

at 9 p.m. had difficulty in converting glucose to fructose. When given fructose, however, they made glucose, which may explain why their synthetic efficiency when given fructose was higher than when given glucose at 9 p.m.

These results suggest that some component of the mechanism of conversion of glucose to fructose and formation of sucrose is less active in blades detached from the plant at night than in blades detached from the plant during the day, but that this component is active again in blades detached from the plant in the early morning before dawn. Darkness itself is not the controlling factor, because all these tests are conducted in total darkness, and the results in Fig. 3 show that blades can continue to make sucrose from glucose when kept in total darkness for two weeks. This component may be used up or bound during the day and resupplied or released during the night, or there may be a "circulation" of the component into and out of the blade. In this connection the diurnal migration of phosphorus from bean leaves studied by Biddulph (4) is highly suggestive. Biddulph found no migration of phosphorus from 7 p.m. to midnight, and that the migration of phosphorus both into and out of the leaf was accelerated in the early morning before dawn.

3. Temperature and the formation of sucrose:

Detailed studies of the effect of temperature upon the interconversion of glucose and fructose and the formation of sucrose have already been published in full (35). The effect of temperature upon the daily percentages of sugars in sugar cane blades supplied with glucose is graphed in Fig. 4, and in blades supplied with fructose in Fig. 5. At 6°C. the blades absorbed the sugar supplied but there was little conver-

sion to the other reducing sugar and little formation of sucrose. But at 20°C. most of the sugar absorbed was converted into sucrose. At 30°C. and 40°C. there was a greater accumulation of sucrose than at the lower temperatures, and also both glucose and fructose accumulated. The higher temperatures increased the absorption of sugar, as shown by the percentages of total sugars. The synthetic efficiency was

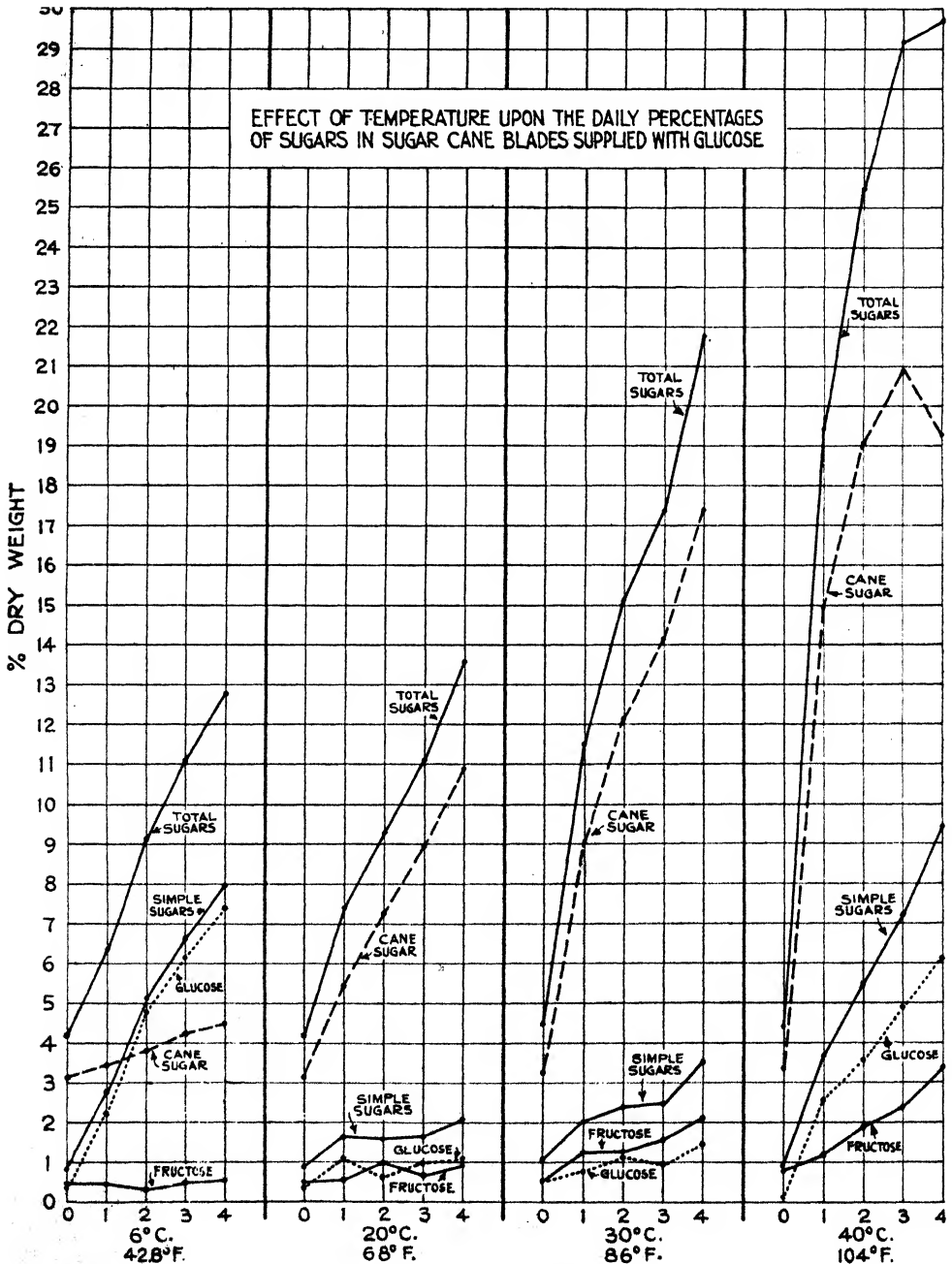


Fig. 4

best at 30°C. These results show that temperature affects the absorption of sugar, the interconversion of glucose and fructose, and the formation of sucrose.

4. Chlorophyll and the formation of sucrose:

Partially and completely albino stalks occasionally develop in stools of cane in the field. One completely albino stalk and one green stalk from the same stool of cane, variety POJ 2878, were used in a test reported in 1937 (33), which showed that albino blades can make sucrose from glucose exactly as well as green blades.

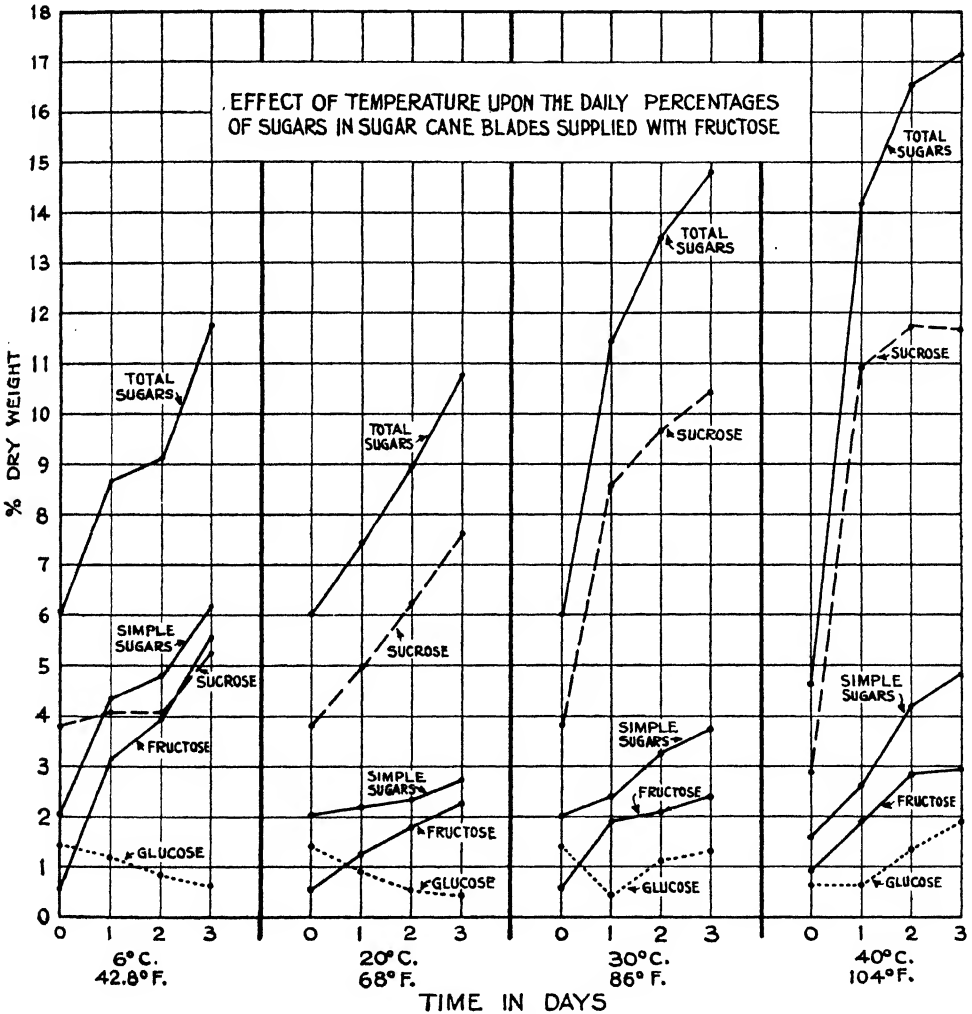


Fig. 5

This experiment was repeated using blades from six albino stalks and six green stalks, using cross No. 5128. In one test the blades were supplied with glucose and in another test, using ratoons, they were supplied with fructose. The results are presented in Table XII. The synthetic efficiencies of the blades supplied with glucose were as follows: albino, 70.74; green, 55.61. The synthetic efficiencies of the blades

TABLE XII

**MOISTURE AND SUGAR PERCENTAGES IN ALBINO AND GREEN BLADES
OF CROSS NO. 5128, SUPPLIED WITH GLUCOSE OR FRUCTOSE FOR 24 HOURS**

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control:				
Albino	79.98 ± 0.224	0.975 ± 0.040	1.092 ± 0.030	2.124 ± 0.008
Green	77.06 ± 0.200	1.029 ± 0.005	2.027 ± 0.022	3.163 ± 0.028
Supplied with glucose:				
Albino	78.25 ± 0.219	3.537 ± 0.011	7.150 ± 0.065	10.687 ± 0.053
Green	76.14 ± 0.358	6.020 ± 0.084	8.723 ± 0.035	15.202 ± 0.121
Initial control:				
Albino	71.20 ± 0.029	0.952 ± 0.003	1.311 ± 0.001	2.332 ± 0.003
Green	68.58 ± 0.057	0.557 ± 0.033	4.630 ± 0.035	5.431 ± 0.004
Supplied with fructose:				
Albino	68.65 ± 0.296	2.005 ± 0.031	2.772 ± 0.076	4.923 ± 0.049
Green	63.91 ± 0.224	2.966 ± 0.014	5.012 ± 0.033	8.242 ± 0.049

supplied with fructose were as follows: albino, 56.39; green, 13.59. The albino blades made sucrose even better than the green blades.

The synthetic efficiencies of the green blades are the lowest ever obtained with apparently normal green blades in the absence of inhibitory substances. No explanation can be offered except that a new cross was used.

Since blades totally lacking in chlorophyll can make sucrose from either glucose or fructose, it is obvious that chlorophyll plays no part in the synthesis of sucrose. Roots are also totally devoid of chlorophyll, but excised roots readily make sucrose when aerated, a point to be discussed in another section. Since neither chlorophyll nor light is required for the formation of sucrose, the process of the synthesis of sucrose must be distinct from photosynthesis, which, however, is essential for it supplies the raw materials for the formation of sucrose.

In another experiment, etiolated shoots of variety H 109 were used instead of albino blades. The percentages of moisture and sugars are presented in Table XIII, the gains in sugars and the synthetic efficiencies in Table XIV, and the percentages of fructose and glucose in Table XV. It is true that the etiolated shoots made

TABLE XIII

**MOISTURE AND SUGAR PERCENTAGES IN ETIOLATED SHOOTS SUPPLIED
WITH GLUCOSE OR FRUCTOSE FOR 24 HOURS**

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	88.78 ± 0.009	10.142 ± 0.092	3.15 ± 0.052	13.46 ± 0.038
Glucose	86.70 ± 0.253	11.203 ± 0.077	4.45 ± 0.000	15.88 ± 0.076
Fructose	86.98 ± 0.081	11.799 ± 0.006	3.69 ± 0.009	15.69 ± 0.019

TABLE XIV

**GAINS IN SUGARS AND SYNTHETIC EFFICIENCY IN ETIOLATED SHOOTS
SUPPLIED WITH GLUCOSE OR FRUCTOSE FOR 24 HOURS,
CALCULATED FROM TABLE XIII**

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	2.42	1.30	53.71
Fructose	2.23	0.54	24.21

TABLE XV
FRUCTOSE AND GLUCOSE PERCENTAGES IN ETIOLATED SHOOTS
SUPPLIED WITH GLUCOSE OR FRUCTOSE FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	4.592 \pm 0.000		5.550 \pm 0.092	
Glucose	5.497 \pm 0.031	0.905	5.706 \pm 0.046	0.156
Fructose	5.112 \pm 0.029	0.520	6.686 \pm 0.035	1.136

sucrose. However, the synthetic efficiencies with either glucose or fructose were lower than generally obtained with variety H-109 under normal conditions.

The results for fructose and glucose are of considerable interest, for in the shoots supplied with glucose there was an accumulation of fructose; and in the shoots supplied with fructose there was an accumulation of glucose. Evidently the interconversion of glucose and fructose could take place readily in these etiolated shoots.

Several attempts were made to repeat this experiment with etiolated shoots, but without success. The etiolated shoots were already so high in sugars (chiefly reducing sugars) that they seldom could be induced to take up more.

5. *Aeration and the formation of sucrose:*

The importance of aeration for interconversion and synthesis was studied in both blades and roots. The effect of aeration in blades was studied by submerging some of the blades in solutions of glucose or fructose. Air was supplied to the aerated series by being pumped through aerators. Several types of aerators were used, and the most satisfactory was found to be pressure tubing punctured uniformly 48 times with an ice pick. The amount of aeration was varied by using one or more aerators per series. The results of one of the experiments with blades are recorded in Table XVI. The gains in total sugars and sucrose and the synthetic efficiency are shown in Table XVII. The blades deprived of aeration absorbed some sugar but lost sucrose, while the blades which were aerated absorbed more sugar and made a little

TABLE XVI
MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH
5% GLUCOSE OR FRUCTOSE WITH AND WITHOUT AERATION

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	68.10 \pm 0.048	0.602 \pm 0.025	2.205 \pm 0.023	2.924 \pm 0.001
Emergent blades:				
In glucose	68.42 \pm 0.019	1.525 \pm 0.023	6.503 \pm 0.071	8.370 \pm 0.052
In fructose	67.86 \pm 0.052	1.701 \pm 0.026	6.073 \pm 0.027	8.094 \pm 0.002
In both	68.08 \pm 0.019	1.355 \pm 0.016	6.125 \pm 0.006	7.802 \pm 0.022
Submerged—not aerated:				
In glucose	71.27 \pm 0.019	2.108 \pm 0.008	1.609 \pm 0.035	3.802 \pm 0.028
In fructose	70.51 \pm 0.038	1.515 \pm 0.016	1.792 \pm 0.002	3.400 \pm 0.013
In both	69.97 \pm 0.048	1.279 \pm 0.002	1.955 \pm 0.012	3.338 \pm 0.011
Submerged—aerated:				
In glucose	70.69 \pm 0.043	1.273 \pm 0.003	2.719 \pm 0.025	4.135 \pm 0.023
In fructose	71.59 \pm 0.081	1.658 \pm 0.000	3.157 \pm 0.011	4.982 \pm 0.011
In both	70.62 \pm 0.048	1.367 \pm 0.013	3.668 \pm 0.005	5.223 \pm 0.019

TABLE XVII

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH 5% GLUCOSE OR FRUCTOSE WITH AND WITHOUT AERATION, CALCULATED FROM TABLE XVI

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Emergent blades:			
In glucose	5.446	4.298	78.92
In fructose	5.170	3.868	74.81
In both	4.878	3.920	80.36
Submerged—not aerated:			
In glucose	0.878	—0.596	0
In fructose	0.476	—0.413	0
In both	0.414	—0.250	0
Submerged—aerated:			
In glucose	1.211	0.514	42.44
In fructose	2.058	0.952	46.25
In both	2.305	1.463	63.47

sucrose. Synthesis was not as efficient in the submerged, aerated blades as in the emergent blades, as shown by the synthetic efficiency.

The effect of aeration upon synthesis in roots is shown in Table XVIII. The gains in total sugars and sucrose and the synthetic efficiency are shown in Table XIX. The excised roots with no aeration absorbed glucose but made no sucrose.

TABLE XVIII

MOISTURE AND SUGAR PERCENTAGES IN EXCISED ROOTS SUPPLIED WITH 5% GLUCOSE, WITH DIFFERENT AMOUNTS OF AERATION

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	87.24 ± 0.086	1.402 ± 0.060	2.662 ± 0.007	4.204 ± 0.052
Glucose:				
No aerators	89.07 ± 0.133	6.590 ± 0.024	1.676 ± 0.023	8.355 ± 0.000
1 aerator	89.08 ± 0.157	7.873 ± 0.002	10.443 ± 0.076	18.866 ± 0.078
2 aerators	86.75 ± 0.215	6.621 ± 0.040	11.726 ± 0.127	18.965 ± 0.093
3 aerators	86.80 ± 0.129	6.122 ± 0.026	13.218 ± 0.005	20.035 ± 0.030
6 aerators	86.13 ± 0.100	5.907 ± 0.016	13.064 ± 0.038	19.659 ± 0.056

TABLE XIX

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF ROOTS SUPPLIED WITH 5% GLUCOSE, WITH DIFFERENT AMOUNTS OF AERATION, CALCULATED FROM TABLE XVIII

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
No aerators	4.151	—0.986	0
1 aerator	14.662	7.781	53.06
2 aerators	14.761	9.064	61.40
3 aerators	15.831	10.556	66.67
6 aerators	15.455	10.402	67.30

When aerated, they absorbed much more glucose and made considerable sucrose. Increasing the number of aerators from one to three increased both absorption and synthesis, but increasing the number of aerators from three to six had very little effect.

The results for glucose and fructose are reported in Table XX. In the series

TABLE XX

FRUCTOSE AND GLUCOSE PERCENTAGES IN EXCISED ROOTS SUPPLIED WITH 5% GLUCOSE, WITH DIFFERENT AMOUNTS OF AERATION

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	1.160 \pm 0.160		0.241 \pm 0.101	
No aerators	1.709 \pm 0.018	0.549	4.881 \pm 0.006	4.640
1 aerator	1.865 \pm 0.040	0.705	6.008 \pm 0.039	5.767
2 aerators	2.421 \pm 0.061	1.261	4.200 \pm 0.101	3.959
3 aerators	2.255 \pm 0.019	1.095	3.866 \pm 0.045	3.625
6 aerators	2.698 \pm 0.048	1.538	3.209 \pm 0.031	2.968

with no aerators, all of the glucose absorbed remained as glucose in the roots, since the small gain in fructose could be accounted for by the loss in sucrose. With aeration there were greater gains in fructose as well as considerable gains in glucose. Evidently aeration is essential for the conversion of glucose to fructose. Aeration is required not only for the conversion of glucose to fructose but also for the synthesis of sucrose, since blades supplied with both glucose and fructose made no sucrose unless aerated. The exact rôle of aeration is not apparent from this test. Aeration may be needed for the formation of an intermediate compound, or for the release of energy required for synthesis, or both. This question will be discussed later.

Oxygen may be supplied to roots by the use of hydrogen peroxide, without forced aeration. Zimmerman (85) found that hydrogen peroxide is good for rooting cuttings. In the following experiment, 60 cc. of 3 per cent hydrogen peroxide were used per liter of 5 per cent glucose. The results are recorded in Table XXI. The gains in sugars and the synthetic efficiency are shown in Table XXII. Some

TABLE XXI

5% GLUCOSE AND OXYGEN FROM H₂O₂

MOISTURE AND SUGAR PERCENTAGES IN EXCISED ROOTS SUPPLIED WITH

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	89.05 \pm 0.091	2.707 \pm 0.009	3.034 \pm 0.030	5.900 \pm 0.040
Glucose—3 aerators	86.88 \pm 0.105	5.859 \pm 0.019	14.845 \pm 0.022	21.486 \pm 0.042
Glucose—H ₂ O ₂	89.53 \pm 0.143	9.939 \pm 0.022	4.151 \pm 0.000	14.309 \pm 0.022
Glucose—H ₂ O ₂	89.58 \pm 0.243	9.851 \pm 0.040	4.214 \pm 0.019	14.287 \pm 0.019

TABLE XXII

GAINS IN SUGARS AND THE SYNTHETIC EFFICIENCY IN EXCISED ROOTS SUPPLIED WITH 5% GLUCOSE AND OXYGEN FROM H₂O₂, CALCULATED FROM TABLE XXI

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose—3 aerators	15.586	11.811	75.77
Glucose—H ₂ O ₂	8.409	1.117	13.28
Glucose—H ₂ O ₂	8.387	1.180	14.06

sugar was absorbed and some sucrose made by the roots supplied with oxygen from hydrogen peroxide, but neither absorption nor synthesis was as good as when oxygen was supplied by forced aeration.

To recapitulate, aeration increases the absorption of glucose and is absolutely essential for the conversion of glucose into fructose and for the synthesis of sucrose.

6. *Mutilation and the formation of sucrose:*

The object of these tests was to find the effects of cutting and grinding upon the formation of sucrose from glucose. In the first experiment the blades were divided into thirds—the lower, middle, and upper third—and were then placed with their lower ends in 5 per cent glucose for 24 hours. The results are presented in Table XXIII. The gains in sugars and the synthetic efficiencies are recorded in Table XXIV which shows that the lower third of the blade was the most efficient in synthesis and the upper third of the blade absorbed the most sugar. The middle third of the blade was the poorest in both absorption and synthesis.

TABLE XXIII
MOISTURE AND SUGAR PERCENTAGES IN LOWER, MIDDLE, AND UPPER
THIRDS OF BLADES, SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control:				
Entire	74.15 ± 0.172	1.338 ± 0.005	2.037 ± 0.001	3.483 ± 0.003
Lower third	77.84 ± 0.062	2.008 ± 0.011	3.420 ± 0.001	5.608 ± 0.012
Middle third	73.45 ± 0.215	1.125 ± 0.027	1.626 ± 0.008	2.837 ± 0.017
Upper third	68.73 ± 0.038	0.802 ± 0.008	1.003 ± 0.002	1.859 ± 0.010
In 5% glucose:				
Entire	71.07 ± 0.091	2.259 ± 0.006	6.484 ± 0.022	9.085 ± 0.017
Lower third	77.72 ± 0.200	2.127 ± 0.029	5.385 ± 0.009	7.796 ± 0.019
Middle third	71.54 ± 0.057	1.637	2.553	4.324
Upper third	66.35 ± 0.091	2.683 ± 0.011	6.219 ± 0.003	9.230 ± 0.015

TABLE XXIV
GAINS IN SUGARS AND SYNTHETIC EFFICIENCIES OF LOWER, MIDDLE, AND
UPPER THIRDS OF BLADES SUPPLIED WITH 5% GLUCOSE FOR
24 HOURS, CALCULATED FROM TABLE XXIII

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Entire	5.602	4.447	79.38
Lower third	2.188	1.965	89.81
Middle third	1.487	0.927	62.34
Upper third	7.371	5.216	70.76

The effect of dividing the blade into midribs and laminae was next studied, with the results recorded in Table XXV. The gains in sugars and the synthetic efficiencies are tabulated in Table XXVI which shows that the laminae are more efficient in both absorption and synthesis than the midribs. The percentages of fructose and glucose are reported in Table XXVII which shows a tendency toward the accumulation of glucose in the midribs but not in the laminae. This indicates that the conversion of glucose to fructose takes place better in the laminae than in the midribs.

TABLE XXV

MOISTURE AND SUGAR PERCENTAGES IN MIDRIBS AND LAMINAE SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control:				
Entire	72.20 \pm 0.000	0.970 \pm 0.000	1.644 \pm 0.003	2.701 \pm 0.004
Midribs	74.71 \pm 0.172	1.516 \pm 0.005	2.115 \pm 0.018	3.742 \pm 0.013
Laminae	67.99 \pm 0.005	0.490 \pm 0.023	1.120 \pm 0.026	1.670 \pm 0.004
In 5% glucose:				
Entire	71.02 \pm 0.024	1.298 \pm 0.001	5.133 \pm 0.025	6.702 \pm 0.027
Midribs	73.95 \pm 0.019	2.732 \pm 0.007	4.845 \pm 0.011	7.832 \pm 0.004
Laminae	67.52 \pm 0.129	1.220 \pm 0.018	5.753 \pm 0.020	7.277 \pm 0.003

TABLE XXVI

GAINS IN SUGARS AND SYNTHETIC EFFICIENCIES OF MIDRIBS AND LAMINAE SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS, CALCULATED FROM TABLE XXV

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Entire	4.001	3.489	87.20
Midribs	4.090	2.730	66.74
Laminae	5.607	4.633	82.62

TABLE XXVII

FRUCTOSE AND GLUCOSE PERCENTAGES IN MIDRIBS AND LAMINAE SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control:				
Entire	0.344 \pm 0.011		0.626 \pm 0.010	
Midribs	0.862 \pm 0.023		0.654 \pm 0.017	
Laminae	0.463 \pm 0.004		0.042 \pm 0.020	
In 5% glucose:				
Entire	1.202 \pm 0.020	0.858	0.096 \pm 0.019	-0.530
Midribs	1.076 \pm 0.051	0.214	1.656 \pm 0.058	1.002
Laminae	0.674 \pm 0.058	0.211	0.546 \pm 0.040	0.504

The effect of cutting the blades into pieces approximately one inch in length was next studied. The blades were cut with scissors. Preliminary tests showed that blades cut in this manner and submerged in 5 per cent glucose with no aeration made little or no sucrose. Aeration enabled the cut blades to make sucrose, but the synthetic efficiency of the cut blades was not as great as that of the entire emergent blades. To find out whether it was cutting or submerging the blades that decreased synthesis, intact and cut blades, submerged, with and without aeration were then compared. The results are recorded in Table XXVIII. The gains in sugars and the synthetic efficiencies are reported in Table XXIX. Since the synthetic efficiency of the cut blades, aerated, was as high as that of the intact blades, aerated, it is evident that cutting the blades into pieces approximately the size of one inch does not decrease their ability to make sucrose from glucose.

Since cutting the blades did not decrease synthesis, the effect of grinding with the Buffalo Cutter was then studied. After 24 hours in 5 per cent glucose, aerated, the cut blades were washed in a wire basket, dried superficially by centrifuging, ground, and sampled. The ground blades were placed in cheesecloth in a wire

TABLE XXVIII

MOISTURE AND SUGAR PERCENTAGES IN INTACT AND CUT BLADES SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS, WITH AND WITHOUT AERATION

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	72.85 ± 0.071	1.586 ± 0.001	2.516 ± 0.011	4.234 ± 0.013
In 5% glucose;				
Intact emergent	68.53 ± 0.057	2.677 ± 0.011	10.795 ± 0.013	14.041 ± 0.025
Intact submerged,				
not aerated	73.66 ± 0.081	2.631 ± 0.005	1.830 ± 0.001	4.557 ± 0.006
Intact submerged,				
aerated	72.96 ± 0.052	2.496 ± 0.012	4.745 ± 0.001	7.491 ± 0.013
Cut, not aerated	73.70 ± 0.071	3.077 ± 0.011	1.248 ± 0.092	4.391 ± 0.086
Cut, aerated	73.72 ± 0.009	2.246 ± 0.001	4.330 ± 0.011	6.804 ± 0.010

TABLE XXIX

GAINS IN SUGARS AND SYNTHETIC EFFICIENCIES IN INTACT AND CUT BLADES SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS, WITH AND WITHOUT AERATION, CALCULATED FROM TABLE XXVIII

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Intact emergent	9.807	8.279	84.41
Intact, submerged, not aerated	0.323	-0.686	0
Intact, submerged, aerated ...	3.257	2.229	68.43
Cut, not aerated	0.157	-1.268	0
Cut, aerated	2.570	1.814	70.58

basket, washed, centrifuged in cheesecloth, ground further, and sampled. The results are presented in Table XXX. The gains in sugars and the synthetic efficiencies are shown in Table XXXI which shows that although the ground blades gained in total sugar they made absolutely no sucrose.

The suggestion arose that the ground blades may have made sucrose, but that the sucrose was removed by washing. To determine this point, a test was conducted in which the ground blades were not washed, but were analyzed along with all the glucose supplied. The results are presented in Table XXXII which shows that the ground blades made no sucrose.

TABLE XXX

MOISTURE AND SUGAR PERCENTAGES IN CUT AND GROUND BLADES SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS WITH AERATION

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	74.48 ± 0.153	1.409 ± 0.005	2.354 ± 0.005	3.888 ± 0.011
Cut, aerated	76.60 ± 0.119	2.871 ± 0.007	4.335 ± 0.007	7.435 ± 0.000
Ground, aerated	77.41 ± 0.029	4.135 ± 0.008	2.152 ± 0.018	6.400 ± 0.011

TABLE XXXI

GAINS IN SUGARS AND SYNTHETIC EFFICIENCIES OF CUT AND GROUND BLADES SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS WITH AERATION, CALCULATED FROM TABLE XXX

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Cut	3.547	1.981	55.85
Ground	2.512	-0.202	0

TABLE XXXII

MOISTURE AND SUGAR PERCENTAGES IN ENTIRE AND GROUND BLADES, AERATED, SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS; THE GROUND BLADES ANALYZED WITH THE GLUCOSE WITHOUT WASHING

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	70.83 \pm 0.029	0.898 \pm 0.008	2.020 \pm 0.007	3.024 \pm 0.000
Entire	69.80 \pm 0.014	1.662 \pm 0.005	6.884 \pm 0.005	8.909 \pm 0.000
Ground	71.74 \pm 0.181	8.711 \pm 0.046	0.531 \pm 0.025	9.271 \pm 0.019

TABLE XXXIII

FRUCTOSE AND GLUCOSE PERCENTAGES IN CUT AND GROUND BLADES SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS, WITH AERATION

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	1.003 \pm 0.006		0.406 \pm 0.012	
Cut	1.112 \pm 0.071	0.109	1.759 \pm 0.079	1.353
Ground	0.724 \pm 0.025	-0.279	3.411 \pm 0.033	3.005

Fructose and glucose percentages in the cut and ground blades are reported in Table XXXIII. The ground blades lost fructose and accumulated glucose, indicating that grinding the blades interfered with the conversion of glucose to fructose.

Summary:

The formation of sucrose from glucose can take place in detached blades, sheaths, stems, and roots of the sugar cane plant. Sucrose synthesis can also take place in entire stalks.

More sucrose accumulated in the stems of entire stalks of cane with leaves attached than in excised cane with leaves removed, indicating that both synthesis *in situ* and translocation from the leaf may be involved in the storage of sucrose in the stem.

When entire plants with roots attached were placed in solutions of glucose, the roots absorbed glucose and accumulated sucrose, but in the tops, only the stems gained a little sucrose.

Because the formation of sucrose from glucose or fructose takes place in absolute darkness and does not require chlorophyll, the synthesis of sucrose is a process distinct from photosynthesis.

Blades taken from different levels on the stem differ in their ability to make sucrose from glucose. The same difference is shown when blades store sucrose as a result of photosynthesis. This suggests that the synthesis of sucrose takes place by the same mechanism whether the glucose is supplied artificially or naturally by the process of photosynthesis.

In hourly studies of blades supplied with glucose or fructose, the percentages of sucrose increased much more than the percentages of reducing sugars. In blades supplied with glucose, the percentages of sucrose increased for nearly two weeks, yet the percentages of reducing sugars fluctuated very little.

Some component of the mechanism of conversion of glucose to fructose and formation of sucrose appears to be less active in blades detached from plants at night than in blades detached from plants during the day. This component is active again in blades detached from plants in the early morning before dawn.

Temperature affects the absorption of sugar, the interconversion of glucose and fructose, and the formation of sucrose. Low temperature ($6^{\circ}\text{C}.$) prevented interconversion and synthesis but did not prevent absorption. Synthesis was most efficient at $30^{\circ}\text{C}.$

The formation of sucrose from glucose or fructose took place as well or even better in completely albino blades as in green blades.

✓ Etiolated shoots when supplied with glucose accumulated fructose, and when supplied with fructose accumulated glucose, indicating that they could carry on the interconversion of glucose and fructose. The production of sucrose was less than usual, in etiolated shoots.

Aeration is essential for the interconversion of glucose and fructose and for the formation of sucrose. Aeration also increases the absorption of sugar.

The lower third of the blade was the most efficient in synthesis and the upper third absorbed the most sugar. The middle third of the blade was the poorest in both absorption and synthesis. The laminae were more efficient than the midribs in absorption of sugar, conversion of glucose to fructose, and synthesis of sucrose.

Cutting the blades into pieces approximately one inch in length did not decrease synthesis, but grinding the blades inhibited synthesis completely, and also inhibited the conversion of glucose to fructose.

Sugar Prices

96° CENTRIFUGALS FOR THE PERIOD
DECEMBER 16, 1942, TO MARCH 15, 1943

Date	Per pound	Per ton	Remarks
Dec. 16, 1942-Mar. 15, 1943.....	3.74¢	\$74.80	Philippines

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Replication: The Safeguard for Uncontrolled Variation

By R. J. BORDEN

Only by a knowledge of the nature and extent of the expected variations in his basic materials can the investigator of sugar cane problems hope to plan his research to identify and separate the chance effects from his purposely applied treatment effects, and thereby arrive at reliable conclusions. Hence we have brought together herein only a few examples, taken from actual data which have been previously recorded, which show some of the variations found in many of the soil and plant analyses which are commonly made; also common variations found in studies of the crop composition and its growth rates, in crusher juice analyses, in cane yields, cane quality, and sugar yields. Then, without discussing reasons for these variations, and without laying down the principles which should govern the selection of duplicate samples but assuming the use of a sound sampling technique and accurate analytical work, and as far as possible avoiding the usage of mathematical expressions and formula, we have sought in this presentation an opportunity to make the investigator aware of the absolute necessity for replication, if his results are to have a real meaning and be truly evaluated for the benefits of the industry.

Quantitative data secured from measurements in biological studies are characterized by great variations, in contrast to the small variations which are found in the more precise sciences of chemistry, physics, and mathematics. Thus the investigator who studies the sugar cane crop must be fully aware of the variations which are involved in his basic material or he is apt to make erroneous assumptions and likely to draw false conclusions, as a result of overlooking the principles and limitations of sampling; for he must make use of samples and from his measurements on these, make sound inferences with respect to the crop from which his samples were taken.

To awaken a fuller appreciation of the extent and nature of some of the uncontrolled or normal variations which are found, we offer the following illustrations. These have been selected as examples, not necessarily of average or typical condi-

tions but rather of variations actually found in measurements which have been made on what had appeared to the eye to be from relatively uniform materials.

VARIATIONS IN SOIL

One of the contributory factors to differences in cane yields and their composition is the variation that exists within the soil of the area where the crop was grown. Thus even under the same environment, with similar influences from sunshine, temperature, wind, and rainfall, we find soil variations of considerable magnitude, and

SOIL VARIATIONS

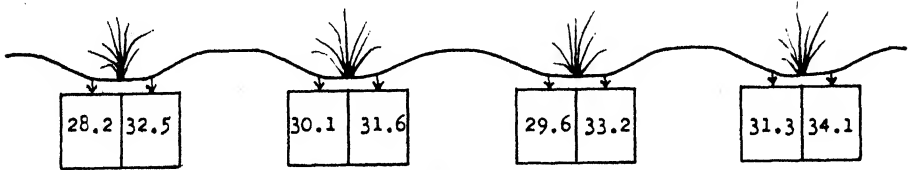


Fig. 1. Percentages of soil moisture at the 6" to 24" depth on opposite sides of 4 adjacent rows of cane.

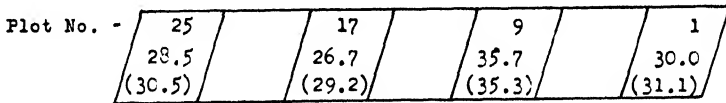


Fig. 2. Percentages of soil moisture in the upper (and second) 12" soil zones of 4 "check" plots.



Fig. 4. Moisture equivalents in adjacent cane rows at 12" deep.

1st. foot:

28.6	34.6	29.6	33.1
20.1	26.7	36.8	29.2

2nd. foot:

29.7	35.5	38.7	62.5
26.6	32.6	47.5	44.8

3rd. foot:

30.7	33.2	62.7	57.1
27.1	35.7	60.5	58.2

Fig. 3. Moisture equivalent values at points 300' apart. Borings from 1st., 2nd., and 3rd. foot of soil

22	27
2.26	2.04
21	26
2.53	2.14
20	25
2.47	2.40

Fig. 5. Per cent total organic matter in upper 12" of soil.

9
12.0
(10.1)
10
8.9
(9.7)
11
11.2
(9.3)

Fig. 6. C/N ratios in upper (and second) foot of soil.

we know that they exert their differential effects upon the growth and composition of a sugar cane crop.

1. *Soil Moisture Percentages:*

Differences as great as 4.3 per cent in soil moisture were recorded within pairs of single auger-bored soil samples taken from the 6-inch–24-inch soil depth on opposite sides of actively growing POJ 2878 cane in adjacent rows of Makiki Field 2 (Fig. 1).

Five days after irrigating the 8 “control” plots in Waipio Experiment 104 I, duplicate soil samples each made up from auger borings taken at two points within each one-tenth acre plot, from both the upper foot and second foot of soil, had variations in their average moisture content between 26.7 and 35.7 per cent (Fig. 2).

2. *Moisture Equivalents:*

Determinations of moisture equivalents of single soil borings taken at the intersections of 300-foot coordinates in Olowalu Field 27 show variations quite characteristic of alluvial soils. In this case the differences are quite large, especially in the samples from the second and third foot of soil (Fig. 3).

Four of the individual soil borings taken 12 inches deep under two adjacent cane rows from Waimanalo Field 11 had moisture equivalents between 41.4 and 37.0 per cent (Fig. 4).

3. *Total Organic Matter:*

The percentages of total organic matter found in samples made up from 10 borings each, taken from the upper foot of soil in 61 plots of Waipio Field L, varied from 1.54 to 2.72. The variation in a small block of six adjacent plots occupying an area of less than three-fourths of an acre ranged between 2.04 and 2.53 per cent—a difference which amounts to nearly 1500 pounds per acre (Fig. 5).

4. *Carbon-nitrogen Ratios:*

The surface foot of soil from each of three adjacent plots, embracing an area of only one-fifth of an acre in Waipio Field L, was found to have a carbon-nitrogen ratio of 11.2, 8.9, and 12.0; the complementary C/N ratios in the second foot of these soils were 9.3, 9.7, and 10.1 respectively (Fig. 6).

5. *Water-soluble Nitrogen:*

Composited soil-bored samples from an irrigated area of growing cane in Kawaihapai Field 1A taken from the upper and the second 12-inch soil layers, both within the row and in the adjoining row-middles, showed a variation in their nitrate nitrogen content which is nicely illustrated in Fig 7. A somewhat different picture, but one still showing a considerable amount of difference in the content of available nitrogen of the upper foot of soil in closely adjoining areas, is shown in Fig 8; here the cane in Grove Farm Field 19A had recently been “hilled up.”

Separate “check” plots in Waipio Experiment V, all included within a total area of less than 1½ acres and adequately sampled at the start of a first ratoon crop, were found to have soil differences in their total water-soluble nitrogen varying from 18 to 85 pounds per acre (Fig. 9).

SOIL VARIATIONS

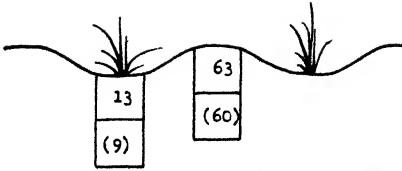


Fig. 7. Pounds of nitrate nitrogen per acre from cane row and adjacent row middle, at 0 - 12" and (12 - 24").

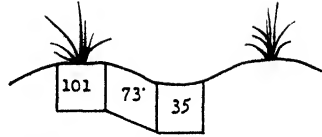


Fig. 8. Pounds per acre of available nitrogen from 3 positions in hilled-up cane.

38		41		21		38		18	
	26		23		18		33		80
85		25		38		38		28	
	26		20		58		33		70

Fig. 9. Pounds per acre of water-soluble nitrogen in soil of "check" plots only (plot size - .038 acre).

3 0	125	100	10 0	15 0
7 0	85	12 5	5 3	10 0
3 8	3 0	7 0	10 0	7 0
3 0	3 8	3 0	8 5	12 5

Fig. 10. Pounds of P_2O_5 per acre from borings at 5 x 5 ft. spacings, after plowing.

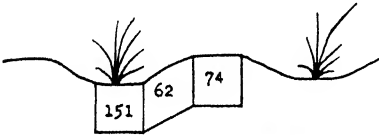


Fig. 11. Pounds P_2O_5 per acre at 3 positions to a depth of 12".

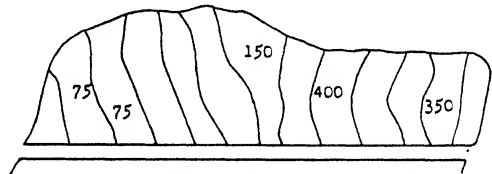


Fig. 12. Pounds of K_2O per acre from 5 sampling stations within a total field area of 21 acres.

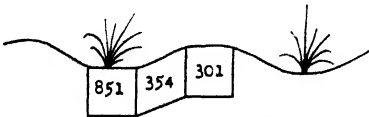


Fig. 13. Pounds of K_2O per acre at 3 positions; cane in the furrow.

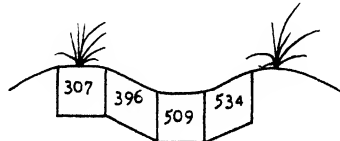


Fig. 14. Pounds of K_2O per acre at 4 positions; cane hilled-up.

6. Available Phosphoric Acid:

Variations in the available phosphate content of continually cropped cane soils are not uncommon. Our example, from single borings made at 5- x 5-foot spacings to a depth of 12 inches in Maui Agricultural Company Field 77, shows a range equivalent to from 30 to 150 pounds P_2O_5 per acre, even after the field had been plowed and prepared for planting (Fig. 10).

Composited auger borings taken to a depth of 12 inches from three positions (with respect to the ratoon cane row) in Lihue Field L26 show a commonly observed condition which is found in ratoon fields, i.e., a higher percentage of available P_2O_5 in the soil in the cane row (Fig. 11).

7. Available Potash:

Reliably taken soil samples from each of 5 level-ditch areas of Pioneer Field H1 covering an area of 21 acres, for purposes of identifying the status of available potash for guidance in the potash fertilization for this field, showed a range between 75 and 400 pounds of K_2O per acre (Fig. 12).

Analyses of soil taken to a depth of 12 inches from Grove Farm Field 17 before fertilizing, from several positions with respect to their proximity to the cane growing in the furrow, showed large variations in the available K_2O content, with higher potash being found directly under the cane row (Fig. 13). However, in Paauhau Field 21 hilled-up cane had somewhat less potash directly under the cane row than farther away (Fig. 14). In both of these cases the soil sampling technique was reliable.

VARIATIONS IN CROP COMPOSITION

A crop of sugar cane, especially after it is a year old, consists of a rather complex aggregation of stalks of different ages and conditions. Its composition is constantly changing, with new stalks being added and old stalks disappearing, and seldom is the extent of these changes predictable. Thus the nature of the crop which is finally harvested can be almost anything than that which was expected earlier.

1. Stalk Census at Harvest:

Something of the variable nature of the stalk population harvested from 10 adjacent 35-foot rows of 31–1389 plant cane which had started its growth with a uniform stand of stalks can be seen from the following record of stalk classes made at the harvest of the Makiki Field 19 Blank Test.

STALK CENSUS, I.E., NUMBER OF STALKS*

CLASSES	ADJACENT ROW NUMBERS									
	12	13	14	15	16	17	18	19	20	21
Sound stalks . . .	186	155	158	144	181	177	175	166	180	186
Dead stalks . . .	3	11	12	36	9	9	25	35	26	9
Suckers	25	5	8	11	21	16	8	16	35	15

* Project A 105—No. 103.

2. Suckers:

The number of suckers with millable cane at harvest is likely to be an extremely variable amount. In Waipio Experiment 108 ATN, suckers with at least 6 feet of millable stalks were counted and weighed in the cane samples taken from some of the "D" plots in October and again in February; their contribution to the total weight of cane harvested varied as indicated below:

SUCKERS AS PER CENT OF TOTAL CANE WEIGHT*

MONTH	DUPLICATE PLOT NUMBERS			
	5	11	32	60
October . . .	9	33	9	33
February . .	14	39	8	37

* Waipio Expt. 108 ATN.

VARIATIONS IN GROWTH

1. *Elongation:*

Those who have studied cane growth and have actually made hundreds of growth measurements are fully aware of the differences in the rates of stalk elongation which apparently similar stalks of cane will often make; those who read the reports which summarize such growth measurements as averages may not always be aware of the extent of these differences in growth which individual stalks of cane make, even under identical external conditions.

From Cornelison's files, we note the following differences in the daily elongation of the first 10 primary stalks of H 109 which he tagged and measured at one growth stage in his studies of cane in the field at Makiki.

PRIMARY STALK ELONGATION (INCHES/DAY)*

TEN DUPLICATE PRIMARY STALKS OF H 109									
No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10
.48	.41	.19	.53	.60	.34	.84	.37	.52	.43

* Data by Cornelison (F.T.G.).

Even when cane is grown under controlled conditions in large pots of the same well-mixed soil and in the same environment, the individual stalks of the same age group will grow at very different rates. Thus primary stalks of POJ 2878 grown in large containers at Makiki showed variations in their daily rates of growth during 4 successive 2-week growth periods as indicated below:

PRIMARY STALK ELONGATION (INCHES/DAY)*

PERIOD: WEEK OF	6 DUPLICATE STALKS OF POJ 2878					
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
1. July 2729	.64	.57	.86	.79	.46
2. August 10 . .	.21	.71	.39	.82	.21	.36
3. August 24 . .	.36	.36	.75	.53	.79	.50
4. September 8 .	.21	.82	.86	.36	.11	.29

* Project A 105—No. 43.

Both of the preceding examples come from measurements made on primary stalks which were tagged for identification in their early growth period and measured periodically thereafter. Hence they may not truly represent the growth being made by the crop, since the crop will include many stalks of a later growth order, i.e., younger stalks which have a considerably faster growth rate. Thus growth measurements made on stalks representative of the entire crop will show an even greater individual variation. This will be apparent from the following tabulation that was made up from measurements on 100 contiguous stalks of H 109 cane grown at Makiki.

COMPLETE POPULATION STALK ELONGATION (INCHES/DAY)*

STALKS	NO. OF STALKS MAKING DAILY GROWTH DURING AUG.-SEPT. OF				TOTAL NO.
	Less than .5"	.5 to .99"	1.0 to 1.49"	More than 1.49"	
All first season . .	5	50	14	0	69
Early second season	0	5	21	5	31

* Project A 105—No. 114.

2. *Density of Stand and Weight per Stalk:*

Records of the density of the stalk population together with the weights of the stalks therein at harvest show variations which at times are the controlling factors in yield differences obtained. An idea of such variations is given in the following record made from 10 adjacent 40-foot rows of cane from plot 1 in the Waipio Field T Blank Test.

STALKS PER FOOT OF ROW AND AVERAGE WEIGHT PER STALK

MEASUREMENT	DUPLICATE ADJACENT ROW NUMBERS									
	1	2	3	4	5	6	7	8	9	10
Number of stalks/ft. . .	4.9	4.1	3.2	3.9	4.1	3.4	2.4	5.2	2.8	3.7
Weight per stalk (lbs.) .	5.8	7.2	9.3	6.2	6.7	7.0	8.2	6.3	9.1	6.3

VARIATIONS IN PLANT COMPOSITION

We are still concerned with pointing out examples of variations in measurements and analyses of duplicated samples taken from the sugar cane crop. We are much more interested in the analysis of the duplicated individual samples than in a duplicate analysis of a composited sample, for much greater variation exists in the sample than in the analytical work. This fact will be better appreciated as we proceed with our examples of variation, many of which are taken from one of our most recent studies in connection with Waipio Experiment 108 ATN.

1. *Per Cent Moisture:*

From 8 replicated plots of Treatment A in Waipio Experiment 108 ATN we can get an idea of the variation in the percentage of moisture found in representative samples of the total green weight by examining the figures secured from the harvest at 10½ months; they range between 72.7 and 77.3 per cent although they come from carefully taken cane crop samples which are duplicates.

PER CENT MOISTURE IN TOTAL GREEN WEIGHTS*

DUPLICATE PLOT NUMBERS							
1	8	13	20	27	28	30	37
76.0	77.3	72.7	72.7	75.4	73.4	73.9	74.7

* Waipio Expt. 108 ATN.

Determinations of the per cent moisture in identical samples of carefully selected leaf sheaths taken from leaves of identical physiological age from cane of the 3 "X" plots of this same field test, during its maximum growth period, reveal differences amounting to 3 and 4 per cent in moisture content.

PER CENT MOISTURE IN LEAF SHEATHS*

DUPLICATE PLOT NOS.	AGE OF CANE (MONTHS)			
	8½	9½	10½	11½
17	71.4	69.6	68.6	69.6
26	70.2	73.7	69.7	71.2
31	73.3	71.8	68.8	72.7

* Waipio Expt. 108 ATN.

2. *Per Cent Reducing Sugars:*

At the age of 14½ months, representative cane samples taken from the 8 replicated plots of Treatment D* showed especially wide variations in their percentages of reducing sugars (dry-weight basis).

PER CENT REDUCING SUGARS IN TOTAL DRY WEIGHTS*

DUPLICATE PLOT NUMBERS							
9	14	19	25	29			
3.86	7.80	8.59	7.07	7.79	8.04	7.67	10.04

* Waipio Expt. 108 ATN.

3. *Per Cent Sucrose:*

The 8 duplicate plots of Treatment C* produced a crop of cane which at 17½ months of age varied from 36.6 to 42.9 per cent in the sucrose concentration of its total dry weight.

PER CENT SUCROSE IN TOTAL DRY WEIGHT*

DUPLICATE PLOT NUMBERS							
3	6	15	18	22	24	33	35
42.9	40.1	41.3	36.6	42.9	41.5	37.4	40.4

* Waipio Expt. 108 ATN.

4. *Per Cent Total Sugars:*

At the October harvest from Waipio Experiment 108 ATN the percentages of total sugars that were found in the total dry weight samples harvested from the 8 replicated plots of Treatment A showed considerable variation—between 36.2 and 46.7 per cent.

PER CENT TOTAL SUGARS IN TOTAL DRY WEIGHT*

DUPLICATE PLOT NUMBERS							
1	8	13	20	27	28	30	37
41.0	45.0	46.7	40.1	41.4	39.9	36.2	38.3

* Waipio Expt. 108 ATN.

* Waipio Expt. 108 ATN.

The concentration of total sugars in the sheaths of active leaves taken from 4 plots of Treatment A was quite dissimilar at some of the earlier preharvests from this same experiment.

PER CENT TOTAL SUGARS IN LEAF SHEATHS*

DUPLICATE PLOT NOS.	AGE OF CANE (MONTHS)			
	7½	8½	9½	10½
23	8.32	9.69	7.76	8.44
28	8.62	9.60	12.42	10.25
34	9.06	11.19	12.92	10.97
37	10.32	12.12	12.47	9.53

* Waipio Expt. 108 ATN.

5. Per Cent Nitrogen:

Cane harvested from a 2 x 5 block of 10 adjacent plots in Waipio Field L, all of which had received identical fertilization at the time the samples were taken, had a variable nitrogen composition between limits of .44 and .72 per cent.

PER CENT NITROGEN IN TOTAL DRY WEIGHT*

DUPLICATE PLOT NUMBERS						
6	12	13	18	19		
.72	.49	.46	.44	.60	.47	.45

* Waipio Expt. 108 ATN.

Four separate leaf-punch samples, taken from 31-1389 cane in rows 2, 5, 8 and 12 of Makiki Field 19 at 12 months, had a composition of 1.59, 1.44, 1.15, and 1.80 per cent nitrogen respectively, in spite of the fact that all this cane had received identical treatment.

The percentage of nitrogen in leaf-punch samples taken from the 7 replicated "C" plots of Waipio Experiment 109 AN at 11½ months varied from 1.45 to 1.77 even though all 7 plots had received identical nitrogen fertilization.

PER CENT NITROGEN IN LEAF-PUNCH SAMPLES*

DUPLICATE PLOT NUMBERS						
41	43	45	48	49	52	53
1.77	1.67	1.67	1.55	1.67	1.45	1.61

* Waipio Expt. 109 AN.

6. Per Cent Chlorophyll in Green Leaves:

Samples of active green-leaf blades, taken from a 2 x 4 block of 8 plots which had been similarly fertilized, showed a range in their chlorophyll content at 5½ months from .075 to .169 per cent.

PER CENT CHLOROPHYLL IN GREEN LEAVES*

DUPLICATE PLOT NUMBERS							
23	24	28	29	33	34	36	37
.075	.129	.158	.127	.145	.127	.169	.127

* Waipio Expt. 108 ATN.

7. *Per Cent Phosphoric Acid:*

An analysis of cane crop samples taken from the H 109 "check" plots of Waipio Experiment 104 I at 10 months showed a difference between the duplicates of as much as 100 per cent in the percentages of phosphate that were found in the total dry-weight samples.

PER CENT P_2O_5 IN TOTAL DRY WEIGHT*

DUPLICATE PLOT NUMBERS							
1	8	9	16	17	24	25	32
.050	.080	.100	.053	.070	.085	.070	.050

* Waipio Expt. 104 I.

At the final harvest at 20½ months of the 8 replicated "A" plots in Waipio Experiment 108 ATN, samples of the total dry weight varied in their P_2O_5 concentration from .132 to .219 per cent.

PER CENT P_2O_5 IN TOTAL DRY WEIGHT*

DUPLICATE PLOT NUMBERS						
13	20	27	28	30	37	
.157	.162	.164	.219	.132	.177	.183

* Waipio Expt. 108 ATN.

8. *Per Cent Potash:*

Variations between .63 and 1.23 were recorded for the percentages of K_2O that were found in truly representative samples of the total dry weight harvested from the 8 "D" plots of Waipio Experiment 108 ATN at 20½ months.

PER CENT K_2O IN TOTAL DRY WEIGHT*

DUPLICATE PLOT NUMBERS						
4	5	9	14	19	25	29
.97	.63	.77	.85	1.23	.87	.73

* Waipio Expt. 108 ATN.

VARIATION IN CRUSHER JUICES

1. *Brix, Pol, and Purity:*

Individual cane samples of 10 entire stalks each, collected at random within a single "burn" of H 109 cane and crushed separately, showed wide variations in their crusher juice analyses.

**BRIX, POL, AND PURITY OF JUICE FROM
DUPLICATE CANE SAMPLES***

MEASUREMENT	DUPLICATE SAMPLE NUMBERS				
	95	96	97	98	99
Brix . . .	18.8	20.7	17.6	16.0	16.2
Pol . . .	15.9	18.2	14.0	11.7	12.2
Purity . .	84.1	87.8	79.7	73.4	75.1

* Project A 105—No. 112.

An extent of the variations found in crusher juices from *all* stalks of 31-1389 plant cane harvested from a few adjacent 30-foot rows in the Makiki Field 19 Blank Test is indicated below:

BRIX, POL, AND PURITY OF CRUSHER JUICES*

MEASUREMENT	ADJACENT ROW NUMBERS					
	3	4	5	6	7	8
Brix . . .	18.0	17.0	17.8	17.6	16.2	15.8
Pol . . .	16.4	15.4	15.8	15.7	14.4	13.5
Purity . .	91.1	90.6	88.8	89.2	88.9	85.4

* Project A 105—No. 103.

Single primary stalks of H 109 cane, which were grown separately in small pots of thoroughly mixed Makiki soil and given identical treatment, produced crusher juices with a considerable amount of variation, even under carefully controlled, identical growth conditions.

BRIX, POL, AND PURITY OF CRUSHER JUICES*

MEASUREMENT	DUPLICATE POT NUMBERS				
	384	389	390	393	397
Brix . . .	21.3	20.0	20.2	19.6	19.0
Pol . . .	20.8	19.0	19.3	17.2	17.8
Purity . .	97.7	95.0	95.5	87.8	93.7

* Project A 105—No. 140.1.

2. *Per Cent Glucose:*

Evidence that differences in the percentage of glucose found in crusher juices from mature cane may be quite considerable is shown by analyses made from duplicate plots of POJ 36 cane harvested at Manoa.

PER CENT GLUCOSE IN CRUSHER JUICES*

DUPLICATE PLOT NUMBERS					
3	6	9	12	15	18
.44	.36	.40	.48	.72	.67

* Project A 105—No. 28.

3. *Per Cent Nitrogen:*

At the July harvest of Waipio Experiment 108 ATN the crusher juices from the 8 replicated plots of Treatment C had a wide variation in their nitrogen content.

PER CENT NITROGEN IN CRUSHER JUICE*

DUPLICATE PLOT NUMBERS							
8	6	15	18	22	24	33	35
.017	.024	.025	.012	.024	.019	.034	.018

* Waipio Expt. 108 ATN.

Even though 31-1389 cane was grown in small pots of well-mixed Makiki soil under identical conditions, the percentages of nitrogen in the crusher juices from 4 duplicate pots at harvest were still quite dissimilar.

PER CENT N IN CRUSHER JUICE*

DUPLICATE POT NUMBERS			
818	819	820	821
.125	.110	.099	.057

* Project A 105—No. 126.

We could cite many examples of this sort from our pot studies—instances where crusher juices from duplicate pots differed by more than 100 per cent.

4. *Per Cent P_2O_5 :*

Six replicated pots of 31-1389 cane grown on Ranch 1 soil without phosphate fertilization produced cane with a different crusher juice concentration of phosphoric acid.

PER CENT P_2O_5 IN JUICE*

DUPLICATE POT NUMBERS					
610	611	612	613	614	615
.060	.045	.096	.056	.044	.080

* Project A 105—No. 122.

Duplicate pots of Manoa soil and also of Makiki soil produced POJ 2878 cane with crusher juice contents of P_2O_5 which were quite different within each soil group.

PER CENT P_2O_5 IN CRUSHER JUICES*

MANOA SOIL DUPLICATES			MAKIKI SOIL DUPLICATES		
No. 51	No. 59	No. 67	No. 78	No. 88	No. 96
.029	.052	.036	.120	.096	.072

* Project A 105—No. 43.

5. *Per Cent K_2O :*

The percentages of potash in crusher juices from 31-1389 cane grown in 5 duplicate pots of Manoa soil showed considerable variation also.

PER CENT K_2O IN JUICE*

DUPLICATE POT NUMBERS				
135	136	137	138	139
.08	.08	.14	.26	.11

* Project A 105—No. 98.

6. *Per Cent N, P_2O_5 , and K_2O :*

This final example is offered to show that even when only 2 primary stalks were allowed to grow in the same pot of Manoa soil, some individual inherent characteristic resulted in crusher juice concentration differences between these two stalks.

PER CENT N, P_2O_5 , K_2O IN CRUSHER JUICES OF
31-1389 CANES*

NUTRIENT	STALK NO.	DUPLICATE POT NUMBERS				
		1293	1294	1322	1327	1329
N . . .	1	.016	.028	.028	.031	.043
N . . .	2	.024	.040	.016	.019	.019
P_2O_5 . .	1	.016	.010	.014	.016	.017
P_2O_5 . .	2	.018	.014	.010	.014	.012
K_2O . .	1	.04	.04	.05	.05	.05
K_2O . .	2	.06	.07	.03	.03	.03

* Project A 105—No. 131.

VARIATION IN CANE YIELDS AND QUALITY

Many of the variations we have just discussed are responsible for the differences so commonly found in actual yields of cane and its quality as harvested from separate units of uniformly treated field areas. In previous discussions we have emphasized these important differences which can occur even on relatively uniform field areas, but it will do no harm to cite again a few examples to complete our picture of normal variations.

Cane Yields:

In Fig. 15 we show the yields of cane (TCA) which were harvested from 8 separate but contiguous .05-acre plots within each of three 5-row strips of Yellow Caledonia plant cane in Pepeekeo Field 20. They tell their own story of the yield variation which existed on what appeared to be a perfectly uniform part of this field, and a careful examination of this area after the yields were recorded revealed no apparent reason for such large differences as were actually found between plot numbers 4 and 5, or 6 and 7, or 10 and 11, or 17 and 18, or 21 and 22.

If further evidence of this nature is desired, another example can be shown by using the cane yields from a block of .05-acre plots from the most uniform-appearing acre of cane in the 1938 Blank Test in Hakalau Field 2A. These are shown in Fig. 16, and though not as "spotty" as the yields shown in Fig. 15, they do illustrate the range in cane yields which we often find even within a small uniform field area.

Variability in yields of cane harvested from duplicate treatments to cane grown in containers of well-mixed soil is a factor for concern for those who use a con-

VARIATIONS IN CANE YIELDS AND QUALITY

1 99	2 97	3 91	4 85	5 66	6 94	7 68	8 67
9 106	10 101	11 82	12 84	13 88	14 70	15 80	16 74
17 86	18 62	19 76	20 76	21 62	22 98	23 89	24 84

Fig. 15. Yields of cane, as tons per acre, from 87' sections of 5-row "X" plots in Pepeekeo Field 20 Blank Test.

64 78	65 93	66 66	67 81	68 92	69 83
34 80	35 81	36 72	37 70	38 66	39 61
4 92	5 83	6 78	7 77	8 82	9 84

Fig. 16. Variations in T.C.A. from a uniform block of .05-acre plots in 1938 Hakalau Blank Test.

2 10.2	15 10.8	28 8.2	41 8.1
3 9.0	16 9.1	29 9.7	42 9.5
4 12.1	17 10.3	30 9.5	43 9.4
5 10.9	18 10.8	31 9.0	44 9.0
6 11.5	19 10.2	32 9.0	45 9.9

Fig. 17. Variation in Y % C in block of 20 plots of 1929 Hakalau Blank Test.

9 9.4	10 9.7	11 8.1	12 8.3	13 8.5	14 9.8	15 10.8	16 11.0
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Fig. 18. Yield % cane variations in 8 sections of a 12-row strip of cane from Grove Farm Expt. 89 V.

trolled "pot-test" technique in their investigations. Thus we have found such yield differences as the following from separate but duplicate small containers cropped under identical conditions.

POUNDS OF CANE HARVESTED FROM POTS HOLDING
5 KILOS OF SOIL*

DUPLICATE POT NUMBERS							
75	80	83	85	88	91	94	96
2.33	2.69	2.77	3.75	2.88	3.73	2.75	1.97

* Project A 105—No. 140.1.

Even with the use of somewhat larger containers, variations in weights, from duplicated pots of cane are apt to be wide:

POUNDS OF CANE HARVESTED FROM POTS HOLDING
8 CUBIC FEET OF SOIL*

DUPLICATE POT NUMBERS								
2	6	10	17	21	25	105	109	113
43	74	57	67	77	70	49	69	60

* Project A 105—No. 123.

Cane Quality:

Differences in cane quality, though perhaps not usually as great as differences in cane yields, are nevertheless found, even when there seems to be no easily apparent difference in the cane itself. Thus in the 1929 Hakalau Blank Test, a block of twenty .05-acre plots located on the most uniform-appearing part of the test area produced Yellow Caledonia cane with quality differences indicated by yield per cent cane values between 8.1 and 12.1 (Fig. 17). All of these plots had received identical treatment. At Grove Farm (Expt. 89V) a uniform 12-row strip of 31-1389 cane, divided into eight .13-acre sections at harvest had variations between 8.1 and 11.0 per cent in its yield per cent cane (Fig. 18).

Cane quality variations are also found even when a controlled pot-culture technique is used by the investigator. Thus 31-1389 cane harvested from 4 large concrete tubs (capacity 500 pounds, soil), all of which had similar exposures to weather factors and identical treatment, showed a considerable amount of difference in their cane quality, even though they differed less than 10 per cent in their cane weights.

YIELD PER CENT CANE*

DUPLICATE POT NUMBERS			
27	31	35	39
15.4	13.7	11.9	14.3

* Project A 105—No. 33.

With smaller containers holding about 70 pounds of well-mixed soil and carefully handled to provide identical growth conditions for the cane therein, variations in the yield per cent cane from 6 pots of 32-8560 cane ranged from 8.3 to 10.9.

YIELD PER CENT CANE*

DUPLICATE POT NUMBERS					
5	13	18	26	40	48
9.1	10.9	10.6	10.0	9.2	8.3

* Project A 105—No. 170.

VARIATION IN SUGAR YIELDS

To complete the picture of variations with which sugar cane investigators must deal, a few instances of the many variations in sugar yields from units of identically treated cane are offered. In Figs. 19 and 20 these are taken from two of the Blank Tests; in Fig. 21 they come from one of the "strip" layouts. Finally, even the carefully controlled pot tests can show such differences between duplicated pots as these.

4	7	14	19	24	29	34	39
10.8	11.1	13.2	11.6	7.3	11.3	10.6	9.6

Fig. 19. Sugar yields, as tons per acre, from middle sections of 500' cane rows in adjacent 10-row plots of Waiialua Blank Test.

43	44	45	46	47	48	49	50	51	52
7.3	10.0	8.3	* 7.9	9.7	10.5	9.7	10.4	* 10.1	8.8

Fig. 20. T.S.A. from block of plots in Hilo Field 19 Blank Test. (* These plots produced identical T.C.A. 81 tons.)

3	7	11	15	19
6.3	8.4	* 6.7	* 7.2	5.4

Fig. 21. T.S.A. from sections of 10-line strip of 31-2306 cane in Grove Farm Expt. 95 V. (* Plots Nos. 7 and 11 had identical cane yields - 65 tons per acre.)

C	B			B	C	C	B	
104	89			88	76	59	60	
	B	C	C	B			B	C
	89	90	80	93			73	83
C	B			B	C	C	B	
99	89			116	99	94	90	

Fig. 22. T.C.A. from replicated plots in Hutchinson Expt. 50.

B plots: Range 60 - 116; average 87.

C plots: Range 59 - 104; average 87.

B 82	C 77
B 84	C 89
C 74	B 87
C 88	B 87
B 101	C 95
B 103	C 85
C 112	B 74
C 91	B 100
B 93	C 97

Fig. 23. T.C.A. from replicated plots in Kekaha Expt. 17.

B plots: Range 70 - 103; Average 91.

C plots: Range 74 - 119; Average 91.

SMALL POTS: POUNDS OF RECOVERABLE SUGAR*

DUPLICATE POT NUMBERS					
32	33	34	35	36	37
.71	.92	.83	.82	.57	.54

* Project A 105—No. 122.1.

LARGE POTS: POUNDS OF RECOVERABLE SUGAR*

5	9	13	DUPLICATE POT NUMBERS			102	106	110
			16	20	24			
7.9	7.9	9.5	8.1	9.9	8.8	6.1	6.7	8.5

* Project A 105—No. 123.

THE SAFEGUARD: REPLICATIONS

If the foregoing discourse upon variations in many of our sugar cane measurements has seemed an unduly long prologue, it is nevertheless an attempt to present critical facts as we have found them. And although such a presentation may seem to have put us up against a rather hopeless and confusing set of actualities, we know that there are certain safeguards which, if adequately and correctly used, will enable us to make sense out of these measurement variations.

The most valuable of these safeguards is repetition or what we more commonly call replication, for this will quite largely distribute these normal variations impartially, and also in such a way that their effects can be identified and measured, if desired. The following examples which are taken from Grade A field experiments will illustrate how a wide range in cane yields harvested from replicated plots which have received identical treatments can "average out" to about the same value when the existing variations are given an unbiased opportunity for impartial distribution to the different treatments. Thus although a series of 9 "B" plots in Hutchinson Experiment 50 produced a wide range of between 60 and 116 tons cane per acre, and their adjacent "C" plots varied between 59 and 104 tons, yet in both series with these wide yield variations the average yield of these 9 plots was the same, i.e., 87 tons per acre (Fig. 22). Similarly from Kekaha Experiment 17 we note that identical average yields of cane were harvested from 9 replicated plots of both Treatment B and Treatment C even though the range in cane yields within each group of 9 plots was extensive (Fig. 23). Many similar instances are on record which show this "evening-up" tendency for normal variations which replication provides. This applies to yield per cent cane values as well as to cane yields. Thus two series of 8 plots each in Ewa Experiment 368K showed a range in Y%C from 11.4 to 13.5 and between 11.2 and 13.7 respectively, yet averaged out to 12.6 and 12.5. And the cane in each of two series of 9 plots from Kaiwiki Experiment 77 averaged 8.5 in Y%C from the 9 separate values which ranged from 6.6 to 9.9 in one series and between 6.8 and 10.1 in the other.

Probably no scientific worker ever actually had too much information or too many measurements from the standpoint of establishing reliable conclusions. No one will argue that a measurement or value from a single sample furnishes as reliable an estimate of the whole as the average value obtained from many samples, *when a proper sampling technique is used*, yet economy of time and labor too frequently limits the number of samples which the investigator is allowed to take. Thereafter, even though his measurements are carefully made with precision instruments and his minutely executed analysis gives him great personal satisfaction, such precision cannot safely be the critique upon which the reliability of his findings is based.

Without an adequacy of samples the accuracy of all hypotheses made therefrom

must suffer. This can be illustrated by an example from Pepeekeo Experiment 50. In Fig. 24 we show the cane yields and relative positions of 10 blocks of plots, each block carrying one plot of each of 3 different treatments, which we have identified simply as X, B, and C, in order to keep this discussion purely objective.

Let us look first at Block No. 1. Here the cane yields from the 3 treatments were identical, and if this had been the full extent of our comparisons we would be

X	B	C	C	B	X
42	42	42	49	44	35
Block 1			Block 2		

C	B	X	X	B	C
44	49	53	57	61	62
Block 3			Block 4		

Block 5			Block 6			Block 7			Block 8		
X	B	C	C	B	X	X	B	C	C	B	X
42	49	59	57	59	57	63	60	60	54	71	63
						C	B	X	X	B	C
						60	55	44	49	50	47
						Block 9			Block 10		

Fig. 24. Pepeekeo Expt. 50. Block and plot identities and plot yields (T.C.A.).

forced to interpret these 42-ton yields as showing no difference in the effect from the 3 treatments. On the other hand if our comparisons had been made only from Block No. 2 our conclusion would have been a very different one, for it would certainly appear that "C" had produced more cane than "B" and that "B" had produced more than "X" in this block. In fact a 14-ton difference between "C" and "X" would have been an easily visible difference and could have been noted from observation alone, both before and at harvest. Thus this example has its word of warning for those who would depend upon single "observation tests," for one would certainly be misled by the information obtained from either one of these blocks alone.

And wait! Look at the yields in Block No. 3 where we now find just the reverse of what we had in Block No. 2, for here the "X" plot has produced more cane than the "B" plot and "B" has produced more than "C." This 9-ton difference between "X" and "C," which here favors "X," was sufficiently large to have been noted by observation, too. Hence, if our comparison, either by observation or by actual yield measurement, had been made only in Block No. 3, our interpretation of treatment effect would have been a still different one.

A further inspection of the comparative yields in the other blocks reveals many further discrepancies. Thus in Blocks No. 4, 6, 7, and 10 the differences between the 3 treatment effects are not very large and they do not consistently favor any one treatment. In Blocks No. 5 and 9 the yields from "C" were greater than from "B" or "X," and "B" was ahead of "X" whereas in Block No. 8 we note "B" better than X or C but here "X" is higher than "C." Confusing? Perhaps so, but problems of a biological source have never been easy to solve, and effects from known differential treatments are not always easy to identify positively because natural variations are so common and extensive and not so easily recognized.

CONCLUSION

We are forced to the conclusion that, in order to allow for the unbiased distribution of natural variation which we have shown to exist in all basic materials which are measured and analyzed by the sugar cane research worker, the most effective way to obtain greater accuracy is to increase the number of replications of samples. We believe that it is far better to have measurements or analyses made from many samples by means of rapid approximate methods than to spend an equivalent time making ultra-refined measurements on only a few samples, because the extent of the variations involved in the sample itself is likely to be much greater than in the measurement concerned, if trained workers are involved in the investigation. But due to a limited labor supply, replication tends to offer us difficulty and the natural tendency is to take fewer but perhaps larger samples or perhaps to composite many carefully obtained samples and thereby reduce the required analytical work. Increasing the unit sample size may help somewhat to increase accuracy but not to the same extent as increasing the replicates, and for comparative purposes much valuable information is lost when separate samples are composited. Therefore, to the careful investigator, replication becomes his fundamental tool, by means of which he obtains a measure of the reliability of his results at the same time that he gets his desired measurements. Thus if he had only one "X" sample and one "Y" sample he could have only one difference and this difference would be a measure of both the treatment effect and the uncontrolled effects. If, however, he had provided several replicates of his X and Y treatment, and designed his investigation to give an effective control of the uncontrolled variations, he can then estimate the amount of influence from these uncontrolled effects and thereafter make a less biased comparison of the actual treatment effects—which is what he actually wants to do.

The Synthesis of Sucrose in the Sugar Cane Plant—II

The effects of several inorganic and organic compounds upon the interconversion of glucose and fructose and the formation of sucrose in detached organs of the sugar cane plant

By CONSTANCE E. HARTT

1. INORGANIC NUTRIENTS AND THE FORMATION OF SUCROSE

Phosphorus:

Six tests have been conducted in which blades were supplied with phosphate along with 5 per cent glucose. In five of these tests the blades with phosphate had better synthetic efficiencies than the blades without phosphate, but in one test the blades with phosphate had a lower synthetic efficiency than the blades without phosphate. The results of one of these tests are presented in Tables I and II, and the results of the other tests are summarized in Table III.

TABLE I

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH 5% GLUCOSE AND DIFFERENT AMOUNTS OF NaH_2PO_4 FOR 24 HOURS

Grams NaH_2PO_4 per liter	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	73.02 \pm 0.033	1.066 \pm 0.009	2.281 \pm 0.000	3.468 \pm 0.008
0	72.37 \pm 0.024	1.565 \pm 0.000	4.598 \pm 0.022	6.406 \pm 0.024
0.4	72.20 \pm 0.076	1.478 \pm 0.002	4.478 \pm 0.003	6.191 \pm 0.000
2.1	72.24 \pm 0.157	1.558 \pm 0.031	4.959 \pm 0.133	6.779 \pm 0.109
8.3	71.49 \pm 0.076	1.687 \pm 0.002	5.371 \pm 0.035	7.341 \pm 0.034

TABLE II

GAINS IN SUGARS AND SYNTHETIC EFFICIENCIES OF BLADES SUPPLIED WITH 5% GLUCOSE AND DIFFERENT AMOUNTS OF NaH_2PO_4 FOR 24 HOURS, CALCULATED FROM TABLE I

Grams NaH_2PO_4 per liter	Gain in total sugars	Gain in sucrose	Synthetic efficiency
0	2.938	2.317	78.86
0.4	2.723	2.197	80.68
2.1	3.311	2.678	80.88
8.3	3.873	3.090	79.78

Plants were grown in aerated nutrient solutions with and without phosphate. At a little less than two months of age, when the tops of the plants supplied with phosphate were twice as tall as those of the plants deprived of phosphate, the plants were

TABLE III

GAINS IN SUGARS AND SYNTHETIC EFFICIENCIES OF BLADES SUPPLIED WITH 5% GLUCOSE OR FRUCTOSE AND 0.8% NaH_2PO_4 OR KH_2PO_4 FOR 24 HOURS

Phosphate supplied	Sugar supplied	Gain in total sugars	Gain in sucrose	Synthetic efficiency
0	Glucose	3.227	2.439	75.58
0	Fructose	2.922	1.930	66.05
0	Both	2.801	2.065	73.72
NaH_2PO_4	Glucose	3.463	3.343	96.53
NaH_2PO_4	Fructose	3.311	3.103	93.71
NaH_2PO_4	Both	3.167	3.169	100.06
0	Glucose	3.198	2.469	77.20
NaH_2PO_4	Glucose	3.364	2.783	82.73
0	Glucose	2.482	1.835	73.93
NaH_2PO_4	Glucose	2.756	1.851	67.16
0	Glucose	2.888	2.152	74.51
NaH_2PO_4	Glucose	3.111	2.438	78.36
KH_2PO_4	Glucose	2.189	1.766	80.67
0	Glucose	4.341	3.487	80.32
KH_2PO_4	Glucose	4.034	3.346	82.94

removed from their solutions, the roots were cut from the tops, and were washed and centrifuged as usual. The excised roots and the entire tops were supplied with 5 per cent glucose for 24 hours. The moisture and sugar percentages are presented in Table IV, the gains in sugars and the synthetic efficiencies in Table V, and the fructose and glucose percentages in Table VI. The roots of the plants grown with

TABLE IV

MOISTURE AND SUGAR PERCENTAGES IN EXCISED ROOTS AND ENTIRE TOPS OF PLANTS GROWN WITH AND WITHOUT PHOSPHATE AND SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control:				
Roots + P	88.38 ± 0.219	1.670 ± 0.014	1.641 ± 0.003	3.397 ± 0.017
Roots - P	87.07 ± 0.005	2.200 ± 0.000	1.286 ± 0.035	3.554 ± 0.036
Tops + P	83.29 ± 0.133	4.421 ± 0.001	3.675 ± 0.023	8.289 ± 0.025
Tops - P	81.16 ± 0.062	2.535 ± 0.063	4.088 ± 0.078	6.839 ± 0.146
In glucose:				
Roots + P	88.48 ± 0.048	7.961 ± 0.028	6.069 ± 0.068	14.350 ± 0.044
Roots - P	87.30 ± 0.009	9.457 ± 0.033	6.351 ± 0.039	16.143 ± 0.008
Tops + P	80.76 ± 0.014	5.441 ± 0.007	4.797 ± 0.032	10.491 ± 0.040
Tops - P	78.16 ± 0.215	4.328 ± 0.032	5.496 ± 0.016	10.114 ± 0.016

TABLE V

GAINS IN SUGARS AND SYNTHETIC EFFICIENCIES IN EXCISED ROOTS AND ENTIRE TOPS OF PLANTS GROWN WITH AND WITHOUT PHOSPHATE AND SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS, CALCULATED FROM TABLE IV

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Roots + P	10.953	4.428	40.42
Roots — P	12.589	5.065	40.23
Tops + P	2.202	1.122	50.95
Tops — P	3.275	1.408	42.99

TABLE VI

FRUCTOSE AND GLUCOSE PERCENTAGES IN EXCISED ROOTS AND ENTIRE TOPS OF PLANTS GROWN WITH AND WITHOUT PHOSPHATE AND SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control:				
Roots + P'	0		1.670 ± 0.014	
Roots — P	1.097 ± 0.005		1.103 ± 0.005	
Tops + P	2.694 ± 0.011		1.726 ± 0.009	
Tops — P	2.289 ± 0.013		0.245 ± 0.077	
In glucose:				
Roots + P	2.044 ± 0.048	2.044	5.917 ± 0.075	4.247
Roots — P	1.754 ± 0.010	0.657	7.703 ± 0.023	6.600
Tops + P	3.251 ± 0.142	0.557	2.190 ± 0.135	0.464
Tops — P	2.545 ± 0.007	0.256	1.783 ± 0.040	1.538

and without phosphate did not differ in synthetic efficiency. But the tops of the plants grown with phosphate had a higher synthetic efficiency than the tops of the plants grown without phosphate. Both the roots and the tops made greater gains in fructose in the plants grown with phosphate, and greater gains in glucose in the plants grown without phosphate. These results suggest that phosphate is important in the conversion of glucose to fructose as well as in the synthesis of sucrose.

In another experiment, in which plants were grown with and without phosphate for three months, detached roots were supplied with glucose in aerated culture for 24 hours, with two replications per series. The synthetic efficiency of the roots grown with phosphate was 34.31 ± 2.499 , while that of the roots grown without phosphate was only 2.92 ± 1.025 .

An experiment was then conducted to determine whether plants grown with and without phosphate for three months would have the synthetic efficiencies of their blades and roots equalized by the addition of phosphate along with the glucose in the synthesis test. For this purpose sodium phosphate and adenylic acid were used. Adenylic acid was chosen because it is an organic phosphate compound and because

studies with other organisms have shown the importance of adenosine triphosphate in the transfer of organic phosphate to glucose. Adenylic acid (= adenosine monophosphate) was obtainable whereas adenosine triphosphate was not. The results of the determinations of moisture and sugars are presented in Table VII, the gains in sugars and the synthetic efficiencies in Table VIII, and the percentages of glucose and fructose in Table IX.

TABLE VII

MOISTURE AND SUGAR PERCENTAGES IN EXCISED ROOTS AND BLADES OF PLANTS GROWN WITH AND WITHOUT PHOSPHATE FOR 3 MONTHS AND SUPPLIED WITH 5% GLUCOSE WITH AND WITHOUT NaH_2PO_4 AND ADENYLIC ACID FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Roots:				
Initial control + P ...	87.31 ± 0.014	1.500 ± 0.002	2.161 ± 0.009	3.776 ± 0.012
Initial control - P ...	85.39 ± 0.033	0.178 ± 0.009	1.125 ± 0.007	1.363 ± 0.003
Glucose + P	87.52 ± 0.105	10.345 ± 0.011	7.737 ± 0.005	18.490 ± 0.017
Glucose + P	88.27 ± 0.272	10.279 ± 0.050	6.758 ± 0.017	17.394 ± 0.068
Glucose - P	87.03	10.323 ± 0.017	2.241 ± 0.008	12.682 ± 0.009
Glucose - P	86.53 ± 0.062	9.949 ± 0.015	1.998 ± 0.005	12.052 ± 0.020
Glucose + NaH_2PO_4 + P	86.78 ± 0.172	8.732 ± 0.005	6.707 ± 0.059	15.793 ± 0.067
Glucose + NaH_2PO_4 - P	87.57 ± 0.009	10.879 ± 0.008	0.926 ± 0.049	11.854 ± 0.059
Blades:				
Initial control + P ...	73.64 ± 0.043	2.381 ± 0.009	2.341 ± 0.016	4.895 ± 0.050
Initial control - P ...	69.15 ± 0.148	0.566 ± 0.015	2.335 ± 0.004	3.024 ± 0.011
Glucose + P	69.44 ± 0.043	6.215 ± 0.047	12.806 ± 0.004	19.695 ± 0.042
Glucose - P	65.26 ± 0.038	3.891 ± 0.037	7.022 ± 0.029	11.283 ± 0.007
Glucose + NaH_2PO_4 + P	70.48 ± 0.133	5.781 ± 0.063	12.089 ± 0.066	18.507 ± 0.133
Glucose + NaH_2PO_4 - P	64.60 ± 0.000	3.919	7.677	12.000
Glucose + adenylic acid + P	69.04 ± 0.129	6.417 ± 0.029	13.438 ± 0.042	20.562 ± 0.015
Glucose + adenylic acid - P	64.53 ± 0.272	4.223 ± 0.014	7.711 ± 0.020	12.340 ± 0.008
Glucose + NaH_2PO_4 + adenylic acid + P ..	69.54 ± 0.081	6.248	12.263	19.157
Glucose + NaH_2PO_4 + adenylic acid - P ..	64.32 ± 0.277	4.056 ± 0.050	7.765 ± 0.056	12.230 ± 0.009

Tables VII and VIII show that the blades and roots of the plants grown with phosphate contained higher percentages of reducing sugars and sucrose than did those of the plants grown without phosphate. The blades and roots of the plants supplied with phosphate also made greater gains in total sugars and sucrose and had higher synthetic efficiencies than did those of the plants deprived of phosphate. Greater gains in glucose and smaller gains in fructose were obtained in the blades and roots of the plants grown without phosphate than in those of the plants grown with phosphate. These findings prove that phosphate plays an important rôle in the conversion of glucose to fructose and the synthesis of sucrose.

TABLE VIII

GAINS IN SUGARS AND SYNTHETIC EFFICIENCIES IN EXCISED ROOTS AND BLADES OF PLANTS GROWN WITH AND WITHOUT PHOSPHATE FOR THREE MONTHS AND SUPPLIED WITH 5% GLUCOSE WITH AND WITHOUT NaH_2PO_4 AND ADENYLIC ACID FOR 24 HOURS, CALCULATED FROM TABLE VII

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Roots:			
Glucose + P	14.714	5.576	37.89
Glucose + P	13.618	4.597	33.75
Glucose — P	11.319	1.116	9.85
Glucose — P	10.689	0.873	8.16
Glucose + NaH_2PO_4 + P	12.017	4.546	37.82
Glucose + NaH_2PO_4 — P	10.491	-0.199	0
Blades:			
Glucose + P	14.800	10.465	70.70
Glucose — P	8.259	4.687	56.75
Glucose + NaH_2PO_4 + P	13.612	9.748	71.61
Glucose + NaH_2PO_4 — P	9.976	5.342	53.54
Glucose + adenylic acid + P	15.667	11.097	70.83
Glucose + adenylic acid — P	9.316	5.376	57.70
Glucose + adenylic acid + NaH_2PO_4 + P	14.262	9.922	69.56
Glucose + adenylic acid + NaH_2PO_4 — P	9.206	5.430	58.98

TABLE IX

FRUCTOSE AND GLUCOSE PERCENTAGES IN EXCISED ROOTS AND BLADES OF PLANTS GROWN WITH AND WITHOUT PHOSPHATE FOR THREE MONTHS AND SUPPLIED WITH 5% GLUCOSE WITH AND WITHOUT NaH_2PO_4 AND ADENYLIC ACID FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Roots:				
Initial control + P	0		1.500 \pm 0.002	
Initial control — P	0		0.178 \pm 0.009	
Glucose + P	1.076 \pm 0.018	1.076	9.269 \pm 0.029	7.769
Glucose + P	1.340 \pm 0.036	1.340	8.939 \pm 0.086	7.439
Glucose — P	0.803 \pm 0.046	0.803	9.520 \pm 0.029	9.342
Glucose — P	0.592 \pm 0.014	0.592	9.357 \pm 0.029	9.179
Glucose + NaH_2PO_4 + P	1.041 \pm 0.017	1.041	7.691 \pm 0.022	6.191
Glucose + NaH_2PO_4 — P	0	0	10.879 \pm 0.008	10.701
Blades:				
Initial control + P	0.503 \pm 0.025		1.880 \pm 0.034	
Initial control — P	0		0.566 \pm 0.015	
Glucose + P	1.672 \pm 0.015	1.172	4.543 \pm 0.062	2.663
Glucose — P	0.651 \pm 0.048	0.651	3.240 \pm 0.010	2.674
Glucose + NaH_2PO_4 + P	1.524 \pm 0.013	1.024	4.257 \pm 0.050	2.377
Glucose + NaH_2PO_4 — P	0.521 \pm 0.047	0.521	3.496	2.930
Glucose + adenylic acid + P	1.581 \pm 0.019	1.081	4.835 \pm 0.009	2.955
Glucose + adenylic acid — P	0	0	4.223 \pm 0.014	3.657
Glucose + adenylic acid + NaH_2PO_4 + P	1.879	1.379	4.369	2.489
Glucose + adenylic acid + NaH_2PO_4 — P	0.629 \pm 0.060	0.629	3.426 \pm 0.009	2.860

Supplying sodium phosphate to the excised blades and roots of the plants grown without phosphate did not increase their synthetic efficiencies, according to Table VIII. Evidently the mere presence of inorganic phosphate is not enough to insure a good ability to make sucrose from glucose. Since phosphate is important in inter-

conversion and synthesis, but inorganic phosphate is not able to make up the deficiency in synthesis tests using plants grown without phosphate, we may conclude that organic phosphate is required for interconversion and synthesis. This conclusion is in accord with the results obtained in an experiment with the enzyme phosphatase, to be presented in another section.

Supplying organic phosphate in the form of adenylic acid to the blades of the plants grown without phosphate resulted in only minor increases in synthetic efficiency, as shown in Table VIII. It is evident that the exact form of organic phosphate required has not yet been found. This subject is treated from a different angle in the third part of this study, dealing with the effects of specific inhibitors upon enzyme action.

Although inorganic phosphate did not increase the synthetic efficiency of the blades of the plants grown without phosphate, there was a small gain in the blades of the plants grown with phosphate, according to Table VIII. This result thus agrees with those already recorded in Tables I-III. Perhaps the addition of inorganic phosphate to the blades of plants grown with phosphate checks the conversion of organic phosphate to inorganic phosphate and thus favors synthesis. This idea is in agreement with the results of the phosphatase tests to be presented in another section. Phosphatase, an enzyme which converts organic phosphate to inorganic, decreases the synthesis of sucrose.

These experiments show that phosphorus is a very important element in the conversion of glucose to fructose and the synthesis of sucrose. It is probable that the form of phosphate required is organic.

Nitrogen:

Blades were taken from plants grown in the field with 0, 100, and 200 pounds nitrogen per acre, and were supplied with 5 per cent glucose for 24 hours. The results for moisture and sugars are reported in Table X, and the gains in sugars and synthetic efficiencies in Table XI. Both the gain in total sugars and the gain in sucrose were negatively correlated with the amount of nitrogen supplied, indicating either that nitrogen interfered with the absorption of sugar, or that some of the sugar absorbed by the blades of the high-nitrogen series reacted with nitrogenous compounds. The synthetic efficiency was best in the blades of the plants grown with 200 pounds N per acre. The activity of invertase was determined in these blades and the results are presented in Table XII. The results are expressed in cc. N/20 KMnO₄ and represent the increase in reducing action using 3 per cent

TABLE X

MOISTURE AND SUGAR PERCENTAGES IN BLADES OF PLANTS GROWN WITH DIFFERENT AMOUNTS OF NITROGEN AND SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control:				
0 N	74.51	1.037 ± 0.012	1.738 ± 0.015	2.866 ± 0.003
100 lbs. N	73.03 ± 0.038	0.968 ± 0.009	1.788 ± 0.014	2.851 ± 0.005
200 lbs. N	74.63 ± 0.043	1.070 ± 0.013	1.739 ± 0.013	2.902 ± 0.000
In glucose:				
0 N	72.98 ± 0.129	1.298 ± 0.002	3.833 ± 0.023	5.333 ± 0.022
100 lbs. N	71.47 ± 0.362	1.172 ± 0.008	3.378 ± 0.043	4.728 ± 0.038
200 lbs. N	72.96 ± 0.095	1.077 ± 0.022	3.045 ± 0.010	4.283 ± 0.010

TABLE XI

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES OF PLANTS GROWN WITH DIFFERENT AMOUNTS OF NITROGEN AND SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS, CALCULATED FROM TABLE X

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
0 N	2.467	2.095	84.92
100 lbs. N	1.877	1.590	84.70
200 lbs. N	1.381	1.306	94.56

TABLE XII

INVERTASE ACTIVITY IN BLADES OF PLANTS GROWN WITH DIFFERENT AMOUNTS OF NITROGEN AND SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS, EXPRESSED IN CC. N/20 KMnO_4

Amount of N	Invertase	
	Initial controls	In glucose
0	15.03	18.38
100 lbs.	17.79	17.24
200 lbs.	18.96	16.57

sucrose at pH 4.5 as the medium. The activity of invertase in the initial controls was positively correlated with the amount of nitrogen, but in the blades supplied with glucose for 24 hours, the activity of invertase was inversely correlated with the amount of nitrogen. In another experiment plants were grown in sand cultures with different amounts of nitrogen and the activity of invertase was determined in separate organs, at pH 4.5, with the results reported in Table XIII. The activity of invertase was positively correlated with the amount of nitrogen in all organs.

TABLE XIII

INVERTASE ACTIVITY IN PLANTS GROWN WITH DIFFERENT AMOUNTS OF NITROGEN, EXPRESSED IN CC. N/20 KMnO_4

Series	Blades	Sheaths	Green-leaf cane	Dry-leaf cane
Complete	17.41	25.87	31.02	50.75
Low N	8.08	13.38	21.29	10.29
No N	7.83	12.71		7.86

Summing up the results with plants grown with different amounts of nitrogen, it appears that both the synthetic efficiency and the activity of invertase were greatest in the plants grown with high nitrogen.

To find the effect of supplying blades with different amounts of nitrogen along with the glucose, experiments were conducted using sodium nitrate and ammonium sulphate. The results of the experiment with sodium nitrate are recorded in Tables XIV to XVI. The synthetic efficiency was increased by the addition of 100-200 p.p.m. N as sodium nitrate, but greatly decreased by the addition of 400-800 p.p.m. N. The conversion of glucose to fructose was also decreased by 400-800 p.p.m. N.

TABLE XIV

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH DIFFERENT AMOUNTS OF NaNO_3 IN 5% GLUCOSE FOR 24 HOURS

p.p.m. N	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	70.96 \pm 0.124	0.949 \pm 0.035	2.737 \pm 0.012	3.832 \pm 0.023
0	70.32 \pm 0.143	2.718 \pm 0.000	7.630 \pm 0.014	10.750 \pm 0.014
100	68.44 \pm 0.148	2.157 \pm 0.002	6.768 \pm 0.010	9.281 \pm 0.009
200	69.58 \pm 0.052	2.231 \pm 0.002	7.128 \pm 0.017	9.734 \pm 0.015
400	68.55 \pm 0.076	2.557 \pm 0.025	5.080 \pm 0.002	7.904 \pm 0.028
800	68.58 \pm 0.153	4.790 \pm 0.008	3.875 \pm 0.007	8.870 \pm 0.015

The results of the experiment with ammonium sulphate are reported in Tables XVII-XIX. Contrary to the findings with sodium nitrate, ammonium sulphate appeared to have no definite effect upon the synthetic efficiency or upon the conversion of glucose to fructose. Since sodium nitrate (400-800 p.p.m. N) decreased the synthetic efficiency, whereas ammonium sulphate (also 400-800 p.p.m. N) had no effect upon the synthetic efficiency, one may conclude either that the deleterious factor in sodium nitrate is the sodium or that the effects of nitrate and ammonium upon synthesis are different. Table II shows that sodium phosphate had no deleterious effect upon synthesis, for which reason it is concluded that sodium is not the inhibitory factor. Therefore these tests indicate that supplying high amounts of nitrate directly to the blades decreased their synthetic efficiency. No tests were made of the actual nitrate content of the blades, but since the detached blades were placed directly in the solutions of nitrate it is possible that the blades contained some nitrate. Normal attached blades, however, growing in the field and supplied with nitrate fertilizer have never been found to contain nitrate, due to the rapid reduction of nitrate which is known to take place in the roots.

TABLE XV

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH DIFFERENT AMOUNTS OF NaNO_3 IN 5% GLUCOSE FOR 24 HOURS, CALCULATED FROM TABLE XIV

p.p.m. N	Gain in total sugars	Gain in sucrose	Synthetic efficiency
0	6.918	4.893	70.72
100	5.449	4.031	73.97
200	5.902	4.391	74.39
400	4.072	2.343	57.53
800	5.038	1.138	22.58

TABLE XVI

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH DIFFERENT AMOUNTS OF NaNO_3 IN 5% GLUCOSE FOR 24 HOURS

p.p.m. N	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.562 \pm 0.032		0.387 \pm 0.003	
0	1.381 \pm 0.155	0.819	1.336 \pm 0.155	0.949
100	1.067 \pm 0.074	0.505	1.089 \pm 0.076	0.702
200	1.053 \pm 0.012	0.491	1.178 \pm 0.009	0.791
400	0.587 \pm 0.006	0.015	1.969 \pm 0.019	1.582
800	0.816 \pm 0.012	0.254	3.974 \pm 0.005	3.587

TABLE XVII

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH DIFFERENT AMOUNTS OF $(\text{NH}_4)_2\text{SO}_4$ IN 5% GLUCOSE FOR 24 HOURS

p.p.m. N	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	67.99 ± 0.071	0.945 ± 0.028	3.161 ± 0.011	4.273 ± 0.016
0	67.09 ± 0.167	1.548 ± 0.033	6.699 ± 0.018	8.601 ± 0.014
100	66.84 ± 0.205	1.293 ± 0.016	5.802 ± 0.037	7.400 ± 0.055
200	66.62 ± 0.014	1.685 ± 0.033	6.367 ± 0.012	8.388 ± 0.046
400	66.31 ± 0.009	1.328 ± 0.002	5.702 ± 0.019	7.330 ± 0.022
800	66.44 ± 0.038	1.493 ± 0.023	5.350 ± 0.005	7.125 ± 0.028

TABLE XVIII

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH DIFFERENT AMOUNTS OF $(\text{NH}_4)_2\text{SO}_4$ IN 5% GLUCOSE FOR 24 HOURS, CALCULATED FROM TABLE XVII

p.p.m. N	Gain in total sugars	Gain in sucrose	Synthetic efficiency
0	4.328	3.538	81.75
100	3.127	2.641	84.46
200	4.115	3.206	77.91
400	3.057	2.541	83.12
800	2.852	2.189	76.75

TABLE XIX

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH DIFFERENT AMOUNTS OF $(\text{NH}_4)_2\text{SO}_4$ IN 5% GLUCOSE FOR 24 HOURS

p.p.m. N	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.488 ± 0.024		0.457 ± 0.004	
0	0.939 ± 0.025	0.451	0.609 ± 0.058	0.152
100	1.067 ± 0.016	0.579	0.226 ± 0.000	-0.231
200	0.956 ± 0.020	0.468	0.729 ± 0.013	0.272
400	0.400 ± 0.003	-0.088	0.927 ± 0.000	0.470
800	0.718 ± 0.004	0.230	0.774 ± 0.019	0.317

These experiments with nitrogen indicate that growing plants with a deficient supply of nitrogen decreases both the activity of invertase and the synthetic efficiency, but that supplying a surplus of nitrate to the blades interferes both with the conversion of glucose to fructose and with the synthesis of sucrose.

2. ORGANIC COMPOUNDS AND THE FORMATION OF SUCROSE

Invertase:

Studies of potassium deficiency in sugar cane reported in 1934 (31)* were thought to offer indirect evidence that sucrose in the sugar cane plant is synthesized by invertase. Oparin (66) claimed to have synthesized sucrose by the simultaneous action of invertase and phosphatase of yeast. Using the method of vacuum infiltration, Kurssanov (48) found that the introduction of very weak concentrations of invertase into leaves stimulated the formation of sucrose from glucose. Studies of the effect of invertase, using "Convertit" prepared by Wallerstein Company were therefore undertaken.

* Numbers in parentheses refer to literature citations at the end of the fourth part of this paper.

When blades were placed in five and ten per cent solutions of Convertit, they absorbed the Convertit which resulted in almost complete inversion of the sucrose already present in the blades. An experiment was then conducted in which the weak concentrations of Convertit suggested by Kurssanov were used. The blades used in this experiment were obtained from plants deficient in nitrogen, since it had already been found that invertase activity is weak in plants deprived of nitrogen. The blades were supplied with Convertit for 24 hours, followed by 5 per cent glucose for 24 hours. The results for moisture and sugars are presented in Table XX,

TABLE XX

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH CONVERTIT FOR 24 HOURS FOLLOWED BY 5% GLUCOSE FOR 24 HOURS

% Convertit	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	68.41 \pm 0.043	0.860 \pm 0.045	2.208 \pm 0.046	3.184 \pm 0.093
0	67.12 \pm 0.200	1.206 \pm 0.009	6.246 \pm 0.006	7.782 \pm 0.016
0.01	68.57 \pm 0.143	1.554 \pm 0.006	6.431 \pm 0.008	8.324 \pm 0.014
0.025	66.98 \pm 0.086	1.294 \pm 0.012	5.840 \pm 0.031	7.442 \pm 0.020
0.05	67.56 \pm 0.076	1.469 \pm 0.045	5.969 \pm 0.034	7.752 \pm 0.009
0.10	67.21 \pm 0.029	1.551 \pm 0.040	5.105 \pm 0.057	6.925 \pm 0.019
0.25	66.53 \pm 0.133	2.230 \pm 0.041	5.200 \pm 0.014	7.703 \pm 0.026

TABLE XXI

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH CONVERTIT FOR 24 HOURS FOLLOWED BY 5% GLUCOSE FOR 24 HOURS, CALCULATED FROM TABLE XX

% Convertit	Gain in total sugars	Gain in sucrose	Synthetic efficiency
0	4.598	4.038	87.8
0.01	5.140	4.223	82.1
0.025	4.258	3.632	85.2
0.05	4.568	3.761	82.3
0.10	3.741	2.897	77.4
0.25	4.519	2.992	66.2

TABLE XXII

INVERTASE ACTIVITY IN BLADES SUPPLIED WITH CONVERTIT FOR 24 HOURS FOLLOWED BY 5% GLUCOSE FOR 24 HOURS, EXPRESSED IN CC. N/20 KMnO₄

Per cent Convertit	Invertase at pH 4.5
Initial control	10.18
0	9.22
0.01	10.73
0.025	13.00
0.05	18.11
0.10	23.04
0.25	38.06

and the gains in sugars and synthetic efficiencies are recorded in Table XXI. The activity of invertase is shown in Table XXII. The activity of invertase in the blades was positively correlated with the percentage of Convertit supplied, indicating that the blades absorbed the Convertit. But the absorption of Convertit did not aid synthesis, as the synthetic efficiencies of all the blades supplied with Convertit were lower than that of the blades with no Convertit. It is true that the blades supplied with 0.01 per cent Convertit made a small but significantly greater gain in sucrose

than the blades with no Convertit, but this was merely due to the fact that they absorbed more glucose, as shown by their greater gain in total sugars.

Only one test has shown an improvement in synthesis in blades supplied with Convertit. The synthetic efficiency of blades without Convertit was 83.0, while that of blades with 0.025 per cent Convertit was 85.4.

The results herein presented are not in accord with the view that invertase synthesizes sucrose. Of course one may argue that the Convertit used was not in the right form. However, it is apparent that one must search for other constituents of the mechanism for the synthesis of sucrose.

Phosphatase:

Phosphatase was prepared from veal marrow bones by the method of Martland and Robison (57). Bone phosphatase hydrolyzes fructose diphosphate forming glucose-, fructose-, and perhaps mannose-6-phosphate, according to MacLeod and Robison (56). Liebknecht (52) reported that bone phosphatase hydrolyzes adenylypyrophosphate (= adenosine triphosphate) splitting off first the easily hydrolyzable phosphate and then the phosphate on the C₅ of the ribose. Our preparation of phosphatase could liberate inorganic phosphate from organic compounds as shown by a test with glycerophosphate. Blades were supplied with approximately 0.25 per cent of the phosphatase preparation for 24 hours, followed by 5 per cent glucose or fructose for 24 hours. The percentages of moisture and sugars are recorded in Table XXIII. The gains in sugars and the synthetic efficiency are shown in Table XXIV.

TABLE XXIII

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH 0.25% PHOSPHATASE FOR 24 HOURS FOLLOWED BY 5% GLUCOSE OR FRUCTOSE FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	72.68 ± 0.033	1.076 ± 0.010	2.766 ± 0.022	3.988 ± 0.033
Water followed by:				
Glucose	71.75 ± 0.029	1.833 ± 0.015	5.250 ± 0.011	7.360 ± 0.027
Fructose	71.39 ± 0.038	2.065 ± 0.019	4.741 ± 0.011	7.055 ± 0.007
Both	69.79 ± 0.019	1.801 ± 0.040	4.876 ± 0.031	6.934 ± 0.007
Phosphatase followed by:				
Glucose	67.74 ± 0.009	2.031 ± 0.012	3.267 ± 0.039	5.471 ± 0.053
Fructose	67.51 ± 0.062	1.856 ± 0.005	3.036 ± 0.014	5.062 ± 0.019
Both	69.38 ± 0.138	2.144 ± 0.019	3.071 ± 0.017	5.377 ± 0.001

TABLE XXIV

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH 0.25% PHOSPHATASE FOR 24 HOURS FOLLOWED BY 5% GLUCOSE OR FRUCTOSE FOR 24 HOURS, CALCULATED FROM TABLE XXIII

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Water followed by:			
Glucose	3.372	2.484	73.66
Fructose	3.067	1.975	64.39
Both	2.946	2.110	71.62
Phosphatase followed by:			
Glucose	1.483	0.501	33.78
Fructose	1.064	0.270	25.37
Both	1.389	0.305	21.95

This experiment indicates that phosphatase hinders synthesis. In another experiment with blades phosphatase decreased the synthetic efficiency, using glucose, from 84.33 to 62.45. In an experiment with excised roots, phosphatase decreased the synthetic efficiency from 39.74 to zero. These results are in accord with the view that organic phosphate plays a rôle in the formation of sucrose.

Invertase and phosphatase are the only enzymes which have been introduced into blades. One experiment was conducted with detached roots supplied with 0.25 per cent zymine in 5 per cent glucose and the result was a decrease in the synthetic efficiency from 39.74 to zero. Other enzymes have been studied indirectly by the use of specific inhibitors, and these studies will be reported in the third paper of this series.

Hormones:

In experiments with entire plants including roots, the roots were found to absorb glucose or fructose and make sucrose; but in experiments with excised roots, the roots were found to absorb glucose or fructose but make little or no sucrose. The suggestion was made (34) that roots obtain from the tops some constituent essential for synthesis. Since aeration has been found to be a very important factor in both interconversion and synthesis, it may be that air is the constituent obtained by roots from the tops. However, even well-aerated roots seldom have a synthetic efficiency equal to that of tops. For this reason, studies of other substances known to be supplied by the tops to the roots were undertaken. The substances studied include hormones and vitamins. Other substances known to be active in lower plants or in animals are also being studied.

The hormones or growth substances used in these tests included beta indole acetic acid, beta indole butyric acid, indole-3-propionic acid, alpha-naphthalene acetic acid, and cinnamic acid. Without exception these substances reduced the synthetic efficiency of excised roots in aerated culture to zero. They also prevented the conversion of glucose to fructose.

Adenylic acid (0.001 per cent) was supplied with 5 per cent glucose in aerated culture to detached roots, and the result was a decrease in synthetic efficiency. Adenylic acid was also used in two tests with blades, resulting in an increase in synthetic efficiency.

Glutathione (0.005 per cent) was used in two tests with detached roots. In one test there was no effect upon the synthetic efficiency, and in the other test there was an increase, but probably insignificant.

Inositol (0.005 per cent) was used in two preliminary tests with detached roots, in both of which there was an increase in synthetic efficiency. However, when a test was conducted with two replications per series, there was no significant difference.

Epinephrine (0.006 per cent), used in one test with detached roots, resulted in a decrease in conversion of glucose to fructose and a decrease in synthetic efficiency. In blades, epinephrine had no effect upon the synthetic efficiency, using glucose (2 tests); in one test, in which fructose was used, epinephrine raised the synthetic efficiency from 81.19 to 88.61.

Alanine (0.005 per cent) was supplied to detached roots in 5 per cent glucose in one test only, and the result was a decrease in synthetic efficiency.

Insulin (0.005 per cent) was supplied to detached roots in 5 per cent glucose in one test only, and the result was a decrease in synthetic efficiency.

Glutamic acid (0.01 per cent) was supplied to detached roots in 5 per cent glucose in two tests, and the result was an increase in synthetic efficiency. There were no significant differences in a test with two replications per series.

Perhaps it should not be surprising that no hormones or growth-promoting substances have been found to increase synthesis consistently. Substances which promote growth would be more apt to stimulate hydrolysis of sucrose and its utilization in respiration and the formation of tissues, than to promote the synthesis of a storage product.

Vitamins:

The following vitamins have been used in tests with detached roots: thiamin chloride, riboflavin, nicotinic amide, pyridoxin, ascorbic acid, 2-methyl-3-phytyl-1, 4-naphthoquinone, the diphosphoric acid ester of dihydro K₁, phthiocol, and folic acid.

Thiamin chloride (0.001 per cent), also known as vitamin B₁, has been used in several tests with detached roots. A preliminary test with a single series indicated that thiamin chloride may aid synthesis. Because of the individual variation in the

TABLE XXV

MOISTURE AND SUGAR PERCENTAGES IN DETACHED ROOTS IN AERATED CULTURE WITH AND WITHOUT THIAMIN CHLORIDE IN 5% GLUCOSE

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	88.82 ± 0.038	2.425 ± 0.007	1.027 ± 0.012	3.506 ± 0.005
Initial control	88.88 ± 0.052	2.516 ± 0.001	0.827 ± 0.001	3.387 ± 0.003
Glucose	89.10 ± 0.033	9.388 ± 0.018	4.259 ± 0.019	13.871 ± 0.038
Glucose	88.72 ± 0.100	9.150 ± 0.020	6.181 ± 0.012	15.656 ± 0.007
Glucose + B ₁	88.40 ± 0.114	9.034 ± 0.007	8.241 ± 0.120	17.709 ± 0.119
Glucose + B ₁	88.39 ± 0.210	8.662 ± 0.019	7.488 ± 0.037	16.544 ± 0.058

TABLE XXVI

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF DETACHED ROOTS IN AERATED CULTURE WITH AND WITHOUT THIAMIN CHLORIDE IN 5% GLUCOSE, CALCULATED FROM TABLE XXV

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	10.425	3.332	31.96 } 37.49 ± 2.638
Glucose	12.210	5.254	
Glucose + B ₁	14.263	7.314	51.27 } 50.68 ± 0.281
Glucose + B ₁	13.098	6.561	

TABLE XVII

FRUCTOSE AND GLUCOSE PERCENTAGES IN DETACHED ROOTS IN AERATED CULTURE WITH AND WITHOUT THIAMIN CHLORIDE IN 5% GLUCOSE

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.899 ± 0.040		1.546 ± 0.023	
Initial control	1.152 ± 0.023		1.364 ± 0.021	
Glucose	1.362 ± 0.008	0.337	8.026 ± 0.026	6.571
Glucose	1.782 ± 0.027	0.757	7.368 ± 0.047	5.913
Glucose + B ₁	1.421 ± 0.153	0.396	7.613 ± 0.146	6.158
Glucose + B ₁	1.105 ± 0.025	0.080	7.557 ± 0.043	6.102

TABLE XXVIII

MOISTURE AND SUGAR PERCENTAGES IN DETACHED ROOTS IN AERATED CULTURE WITH AND WITHOUT RIBOFLAVIN IN 5% GLUCOSE

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	90.07 ± 0.081	1.023 ± 0.055	1.659 ± 0.065	2.769 ± 0.014
Initial control	89.31 ± 0.038	1.452 ± 0.058	1.397 ± 0.000	2.923 ± 0.058
Glucose	90.41 ± 0.024	10.248 ± 0.023	4.781 ± 0.036	15.281 ± 0.061
Glucose	90.86 ± 0.162	11.694 ± 0.013	6.257 ± 0.021	18.280 ± 0.009
Glucose + B ₂	90.76 ± 0.124	11.511 ± 0.028	8.922 ± 0.050	20.903 ± 0.024
Glucose + B ₂	89.55 ± 0.048	10.102 ± 0.011	9.892 ± 0.067	20.514 ± 0.059

TABLE XXIX

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF DETACHED ROOTS IN AERATED CULTURE WITH AND WITHOUT RIBOFLAVIN IN 5% GLUCOSE, CALCULATED FROM TABLE XXVIII

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	12.435	3.253	26.16
Glucose	15.434	4.729	30.64
Glucose + B ₂	18.057	7.394	40.94
Glucose + B ₂	17.668	8.364	47.33

roots, another test was conducted in which each series had two replications, the results of which are presented in Table XXV. Each series had three aerators. The gains in sugars and the synthetic efficiencies are recorded in Table XXVI. The percentages of fructose and glucose are reported in Table XXVII. The results for the synthetic efficiency suggest that thiamin chloride aids synthesis in roots, although the error is high. There is no evidence that thiamin chloride affects the conversion of glucose to fructose. In blades, thiamin chloride has been used in several tests, resulting in some tests in a small increase in synthetic efficiency and in other tests in a small decrease, indicating either that thiamin chloride has no real effect upon synthesis in blades, or that the blades already had enough.

Riboflavin (0.0001 per cent), also called vitamin B₂ or vitamin G, has been used in two tests each with two replications. The results of one of the tests are presented in Table XXVIII. The gains in sugars and the synthetic efficiencies are reported in Table XXIX. The percentages of fructose and glucose are recorded in Table XXX. The synthetic efficiency was significantly greater in the series supplied with riboflavin than in the series with no riboflavin. Similar results were obtained in another replicated test. These results suggest that riboflavin aids synthesis in roots. There is no evidence that riboflavin aids the conversion of glucose

TABLE XXX

FRUCTOSE AND GLUCOSE PERCENTAGES IN DETACHED ROOTS IN AERATED CULTURE WITH AND WITHOUT RIBOFLAVIN IN 5% GLUCOSE

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0		1.023 ± 0.055	
Initial control	0		1.452 ± 0.058	
Glucose	0.358 ± 0.092	0.358	9.890 ± 0.069	8.653
Glucose	0.375 ± 0.084	0.375	11.319 ± 0.071	10.082
Glucose + B ₂	1.552 ± 0.180	1.552	10.459 ± 0.030	9.222
Glucose + B ₂	0	0	10.102 ± 0.011	8.865

to fructose. In blades, the use of riboflavin was sometimes accompanied by a small increase and sometimes by a small decrease in synthetic efficiency.

Nicotinic amide (0.001 per cent), another member of the vitamin B complex, was supplied to detached roots in two tests each with two replications. In the first test the synthetic efficiency of the roots without nicotinic amide was 43.75 ± 0.653 , and with nicotinic amide it was 50.03 ± 0.987 . However, in the second test there were no significant differences in synthetic efficiency with and without nicotinic amide. It would therefore seem that nicotinic amide has no significant effect upon the synthesis of sucrose.

Pyridoxin (0.001 per cent), also called vitamin B₆, was used in two preliminary tests with detached roots. In the first test, the synthetic efficiency of the series without vitamin B₆ was 39.74, and with vitamin B₆, 50.71. In the second test, the synthetic efficiency of the series without vitamin B₆ was 66.97, and with vitamin B₆, 73.72. In a test with two replications per series there was no significant difference in synthetic efficiency with and without vitamin B₆.

Ascorbic acid (0.01 per cent), also named vitamin C, was used in a test with roots with two replications per series. The synthetic efficiency without ascorbic acid was 52.92 ± 0.830 , and with ascorbic acid, 52.47 ± 0.949 .

Water-soluble compounds with vitamin K activity were used in an experiment with detached roots with two replications per series. These compounds were 2-methyl-3-phytyl-1, 4-naphthoquinone and the diphosphoric acid ester of dihydro K₁. The synthetic efficiency without these compounds was 39.26 ± 0.043 ; with the first compound, 40.54 ± 0.391 ; and with the second compound, 42.53 ± 0.997 .

Phthiocol (0.005 per cent) had no significant effect upon the synthetic efficiency of detached roots.

Folic acid (10 gamma per liter) had no significant effect upon the synthetic efficiency of detached roots.

Of the nine vitamins used in tests with detached roots, seven had no significant effect upon synthesis. Replicated tests with thiamin chloride and riboflavin indicated that these vitamins aid the synthesis of sucrose. Although aeration, thiamin chloride, and riboflavin all aid synthesis in detached roots, they do not enable roots to make sucrose as well as blades, which indicates that some factor necessary for synthesis is still deficient in detached roots.

SUMMARY

This paper deals with the effects of phosphorus, nitrogen, and several enzymes, hormones, and vitamins upon the interconversion of glucose and fructose and the formation of sucrose by detached blades and roots of the sugar cane plant supplied with glucose or fructose in the dark.

Results obtained by growing plants with and without phosphate, as well as by supplying detached blades or roots with phosphate along with glucose in the dark, suggest that phosphate plays a rôle both in the conversion of glucose to fructose and in the formation of sucrose.

Bone phosphatase, which liberates inorganic phosphate from organic compounds, decreased or prevented the formation of sucrose from glucose. This suggests that it is organic phosphorus which is essential for the formation of sucrose.

When plants were grown with different amounts of nitrogen, both the synthetic

efficiency and the activity of invertase were greatest in the plants grown with high nitrogen.

When detached blades were supplied with different amounts of sodium nitrate along with glucose in the dark, their synthetic efficiency was increased by the addition of 100-200 p.p.m. N but greatly decreased by 400-800 p.p.m. N. The conversion of glucose to fructose was also diminished by 400-800 p.p.m. N. But when ammonium sulphate was used instead of sodium nitrate, there was no definite effect upon the synthetic efficiency or upon the conversion of glucose to fructose. These results suggest that supplying a surplus of nitrate to the blade may interfere with the interconversion of glucose and fructose and with the formation of sucrose. This does not apply to plants growing in the field, where nitrate fertilizer is rapidly reduced in the roots.

Supplying blades with "Convertit" ranging from 0.01 per cent to 10 per cent resulted in an increased activity of invertase and a decreased ability to make sucrose from glucose.

Growth-promoting substances (beta indole acetic acid, beta indole butyric acid, indole-3-propionic acid, alpha-naphthalene acetic acid, and cinnamic acid) prevented the conversion of glucose to fructose and the formation of sucrose in detached roots supplied with glucose.

The following vitamins were used in replicated tests with detached roots supplied with glucose in the dark, in aerated culture: thiamin chloride, riboflavin, nicotinic amide, pyridoxin, ascorbic acid, 2-methyl-3-phytyl-1, 4-naphthoquinone, the diphosphoric acid ester of dihydro K_1 , phthiocol, and folic acid. Both thiamin chloride and riboflavin, used separately, were found to increase the synthetic efficiency, although the error was high. None of the other vitamins affected synthesis significantly.

Although aeration, thiamin chloride, and riboflavin all aid synthesis in detached roots, they do not enable roots to make sucrose as well as blades, which indicates that some factor necessary for synthesis is still deficient in detached roots.

A Report on 32-8560 at Waialua

By A. C. STEARNS*

The agricultural practices employed in the growing of the old standard varieties such as H 109 and Yellow Caledonia are based upon a wealth of accumulated information developed through the years by experimentation and observation. The advent of a new variety invites a re-examination of established practices. This does not imply that a new beginning must be made; basic principles remain applicable regardless of variety. The revisions in practice required by a new variety involve changes in degree; they are not likely to involve a revision of principles.

Nevertheless, gains of considerable economic importance may be affected by skillful adjustment of agricultural practices to varietal requirements. In the following paper the status of 32-8560 at Waialua Agricultural Company, Ltd., is discussed by A. C. Stearns with the object of recording the information which has been acquired thus far at Waialua, and pointing to questions about which more information is needed.

(A. J. M.)

Introduction:

From an agricultural point of view, the arrival and subsequent success of the sugar cane variety 32-8560 represents a milestone in the progress of Hawaiian sugar agriculture. On irrigated, as well as on a number of unirrigated plantations, this variety is giving outstanding yields and is being spread rapidly. 32-8560 is a result of the intensive cane breeding program that has been carried on by the Experiment Station, H.S.P.A.

32-8560 is the outcome of a cross between the Java cane P.O.J. 2878 and the Indian variety Co. 213. The cross was set up on November 24, 1931, when ten tassels of Co. 213 were placed in a large P.O.J. 2878 crossing rack. Thirty-five seedlings were grown from this cross. The plant crop selection was made on September 22, 1932, by A. J. Mangelsdorf and C. G. Lennox. In this first phase of selection, the seedling received the number 32-8560. The first grading given the seedling was "equal-plus." From the original cross, a total of four seedlings was selected in the plant crop and four more in the ratoons. None of the others survived the preliminary testings and 32-8560 was the only one to arrive definitely as a commercial variety.

The development of 32-8560 at Waialua goes back to 1938. It was in that year that the first commercial field plantings were made. The variety had been planted in experiments, but the results were not available and the small original plantings were made on the basis of experimental results being obtained at other plantations

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and in Experiment Station plantings. From the beginning, 32-8560 was observed to be an unusual variety showing many characteristics that were of particular interest to the field production staff and to management. During the late nineteen thirties, it had become increasingly apparent at Waialua that a variety of sugar cane which was better suited to the upland areas was needed. As these original plantings of 32-8560 progressed, the growth characteristics were noted. The large-scale spreading of 32-8560 was soon underway. The program was started; and with each succeeding year, it increased in intensity and scope. The improvement of field layouts and irrigation efficiency was closely associated with the spreading of this new variety.

With the adoption of a new variety on a commercial basis, field staffs and management are confronted with the problem of understanding how to grow properly the new cane to obtain the maximum economic yields. Generally speaking, most varieties vary in their growth characteristics sufficiently so that it is possible to differentiate in various phases of their cultural treatment. The advantages in learning as soon as possible what the growth characteristics are and how the variety should be grown are obvious. In this report it is my intent to summarize all data that pertain to 32-8560 at Waialua in order to aid in making possible a better understanding of this plant.

Planting of 32-8560 at Waialua:

The development of the present acreage of 32-8560 at Waialua is set forth in the following table:

TABLE I
SUMMARY OF 32-8560 PLANTINGS 1938-1942

Year	Total acres planted	Acres planted to 32-8560	Per cent 32-8560 planted	Total cultivated acres	Per cent 32-8560 of total area
1938	2450.12	101.09	4.13	9828.17	1.03
1939	2845.20	1575.83	55.39	10190.60	16.46
1940	1299.43	776.41	59.75	9662.07	25.39
1941	1944.98	1903.84	97.88	9845.71	44.25
1942	2179.35	2179.28	99.99	8945.38	68.77

Total 32-8560 planted 6536.45 acres (without area losses due to war emergency).

Total 32-8560 planted 6151.49 acres (now in crop).

In 1938 there was about one per cent of the area in 32-8560. In 1939 this increased to approximately 17 per cent. By 1940 this proportion jumped to 25 per cent and by the end of 1941, the percentage was in excess of 44 per cent. With the 1942 planting completed, the present area in 32-8560 is 69 per cent of the total area in cultivation. The last large increase is partially due to a sizable decrease in total area under cultivation. Area losses included some 32-8560 area.

The area planted in 1938 represents such a small amount that it can be said that the spreading of 32-8560 to its present acreage at Waialua was accomplished in a four-year period. This probably represents one of the most rapid changes to a new variety to be found in the history of Hawaiian sugar cane agriculture. If it is possible in the years 1943 and 1944 to complete our scheduled planting programs, we shall be very near to a total cultivated area that will be nearly 100 per cent 32-8560.

What this has meant and will mean to the economy of the Company can be ob-

served in connection with the summary of the yields of 32-8560 through 1942 as shown in Tables VII through X.

Germination and Type of Seed:

The germination of 32-8560 is not too different from that of other varieties. 32-8560 is subject to the same effects of source and type of seed, soil preparation, soil type, time of start, depth of covering, soil moisture as related to the first irrigation and soil temperatures, as well as the effects of seed treatment (handling of seed, dipping with fungicides, elapsed time from cutting of seed to planting, etc.).

In the many plant fields of 32-8560 that have been started at Waialua since 1938, it has been generally observed that the seed germinates in a very satisfactory manner. The plant field stands have been quite even and usually all germination takes place within a month from time of planting. Under favorable conditions, germination is well underway eight to ten days after planting. It proceeds at an increasing rate until fifteen to twenty days after planting and then levels off. On the basis of germination tests that have been conducted in the field, we obtain from 75 per cent to 90 per cent germination based on the number of seed piece eyes actually planted. As will be noted under comments on replant, the amount of replant in 32-8560 fields has been quite low. As was mentioned above, the type of stand that is obtained in plant fields is quite even, although we have noted instances where the condition of the seed used has influenced the evenness of stand. Body seed has been used almost exclusively and has given satisfactory results. Until the 1942 planting season, short seed was used (about 12-15 inches in length). In 1942 it was necessary to handle seed with rope slings instead of burlap bags. It was found that a longer seed piece (20-30 inches) was needed to enable the effective use of slings. The longer seed, in most cases, has given satisfactory stands and the germination has been fairly good.

When planting in the warm summer months, it has been found that it is not necessary to dip seed in Ceresan to obtain good germination; however, during the winter months when soil temperatures are low, it has been found to be an effective and worthwhile practice which aids in obtaining good germination.

Stands of 32-8560, although more rapid in initial growth than H 109, do not appear to grow rapidly until about three to four months after planting. This is a clear-cut phenomena and has been observed in practically all plant fields.

No special effort has been made to develop seed for planting purposes. We have usually selected plant field areas that are from seven to ten months of age and where the prospective seed is of good quality. Although there has been a feeling in some quarters that seed areas should receive careful nitrogen fertilization and irrigation control, we have made no definite move in this direction. Unless seed areas are planned for far in advance, it is not possible to do this effectively. As long as care is exercised in the selection of the seed areas, past experience would indicate that satisfactory results can be obtained.

The seed piece characteristics of 32-8560 are such as to make for easy handling. The eyes are fairly flat and are not damaged to any great extent in transit. Long internodes sometimes make for few eyes per seed piece. This can be considered to be a disadvantage indicating that seed areas should not be allowed to grow too rank. Seed from rank, succulent cane is also subject to more rapid deterioration when placed in contact with the soil.

The amount of seed used per acre in plant fields varies from 50 to 70 bags or bundles per acre depending on the amount of overlap and the size of seed pieces. Size in this instance refers to diameter of the seed piece. Of course, the length of seed piece as related to short seed versus long seed would likewise affect the number of bags or bundles used.

Soil type affects germination to the extent that in adobe soil types where the surface cakes badly after irrigation, the germination is sometimes slower. In the red residual soil types of the Koolau slope, the soil is friable in nature and with other conditions being equal, the rate of germination is usually more rapid than where heavy soils and poor aeration are encountered, as in some of the makai fields.

To insure a favorable germination and stand, the first irrigation follows planting as rapidly as possible. Subsequent irrigations follow at a three- to seven-day interval depending on conditions. As the stand progresses, the interval of irrigation gradually increases up to ten to fourteen days depending on moisture conditions at the time. This is fairly typical of young stands of cane up to six months of age.

Replant—Plant and Ratoons:

Due to the large amount of heavy mechanical equipment that moves through a cane field under present-day operating conditions, the effect on the amount of replant required is evident. This is particularly true of H 109 fields. 32-8560, due to its strong growing characteristics, appears to be a variety that is better suited to the rugged treatment that fields receive under present conditions.

It has been our experience that the amount of replant required in 32-8560 plant fields usually varies between one and two bags per acre. During 1942 in fields where soil preparation has been below optimum and where long seed has been used, we have had instances where replant in plant fields has gone as high as four to six bags per acre. This, however, is considered to be abnormal.

In ratoons of 32-8560 we have found that the amount of replant per acre varies from two to five bags. This refers particularly to first ratoons. In certain instances, second ratoons of this variety have required slightly more than this. 32-8560 is a vigorous ratooner and closes-in more rapidly than plant cane, as would be expected. It is necessary to get the replant in as soon as possible after starting a field; otherwise, the shading-in will eliminate much of the replant as an effective part of the production potential of the field. These replant data in ratoons are based on observations in mechanically harvested fields. All replant seed is dipped in Ceresan solution.

Cultivation:

In many respects the growing characteristics of 32-8560 have a definite appeal to those concerned with the cultivation of the variety. In plant cane, due to its upright growth characteristics, the variety appears to be slow in closing-in as compared with 31-2510, 31-1389 or 31-2806. This is true; however, its yielding ability so overshadows these three mentioned varieties that they cannot be considered in the same class with 32-8560 at Waialua. This openness in plant cane makes 32-8560 somewhat comparable with H 109; however, actually the appearance of millable cane comes earlier with 32-8560. Data indicate first-season growth of

32-8560 to be considerably stronger than H 109. Indications are that ratoons of 32-8560 will be much cheaper to cultivate than the plant crops.

32-8560 due to its vigorous growth is particularly well adapted to mechanical cultivation. Its upright growth can be an advantage here in allowing a close approach to the row of cane. Disc line reshapers and weeders have been used effectively. It has been reported from Ewa that 32-8560 in some instances assumes a partial recumbent form of growth. This has been noted in a minor way at Waialua.

Probably one of the most important phases of field cultivation with particular regard to weeding (hand, spray, and mechanical) is the coordination of the operations. For example, this ties in directly with fertilization where a policy of clean fields prior to fertilizing exists. The basic policy of clean fields prior to fertilizing is sound. The emphasis intended here is that in order to maintain fertilization at the optimum time of application, it is necessary to have a strong coordinated action on the part of field supervision to have the right fields clean at the proper time.

Under Waialua conditions the openness of 32-8560 has not been considered a serious factor. On other plantations this characteristic is of some importance. For instance at Kohala in the mauka areas, the openness is a factor affecting weed control. Other varieties affording more satisfactory weed control and which give comparable yields come into use under such conditions.

Pali-pali and line reshaping operations as they affect 32-8560 ratoons are of importance, particularly in their bearing on irrigation. This ties in closely with the planting and layout of a field since present line reshaping implements are not capable of changing the grade of the original line. To avoid variable growth in ratoons which is attributable to grade of line, shape of line and length of line, and the quality of irrigation resulting from such irregularities, it becomes increasingly important that the layout and planting of fields be closely controlled and supervised. Layout and planting affect cultivation operations as well as irrigation. Both should be developed in such a manner as to aid most effectively in cultivation and irrigation operations.

The quality of plowing and field preparation has been observed to have an effect on subsequent growth and cultivation operations. Factors affecting these operations, particularly labor and equipment are not always controllable; however, it is recognized that good field preparation is worthwhile and has a favorable effect on the crop and cultural operations.

32-8560 and Irrigation Practice:

Irrigation at Waialua is based primarily on control effected through the use of the soil moisture method as adapted and developed by H. R. Shaw and J. Swezey, as well as on experience gained in the irrigation of cane in the several soil types and conditions that exist on the plantation. No special effort has been made to irrigate 32-8560 in any prescribed manner except that good irrigation is stressed, *i.e.*, adequate penetration of water in all parts of each line of cane. Although it has been propounded that 32-8560 is a drought-resistant variety and does not require as many rounds of irrigation as, for example, H 109, we have continued to irrigate it in much the same manner as we do our other varieties.

Based on yields obtained in the past two crops, it would seem that irrigation (a primary control factor in yields at Waialua) was not deviating too far from

optimum. There are several reasons for this as far as 32-8560 is concerned. First, practically all yields are reported from plant fields. Plant fields, due to soil preparation, offer a tilth that affords good conditions for an optimum penetration of water in the cane line. Second, with new plant field installations, field layouts were considerably improved. It is felt that irrigation, as such, has not had a deleterious effect on the yields reported here, except in the Koolau mauka group, because conditions affecting irrigation have been very near to optimum. This refers particularly to yields from plant fields. What will be experienced in the ratoons of 32-8560 may be more closely related to irrigation than the plant field yields. Due to good soil conditions, plant fields overcome some of the irregularities that exist in grade of line and shape of line; however, in ratoon fields where the soil has become quite packed after two years of irrigation, the term tilth no longer applies to the soil. In the ratoon crops, the soil does not permit easy and rapid penetration of water due to the hardness of soil, particularly if grades, shape and length of line are not optimum. In combination, these factors cause irregular distribution of water in the line. It is felt that if comparable irrigation can be obtained in ratoon fields as in plant fields, yields will be maintained reasonably well.

TABLE II
SUMMARY OF IRRIGATION—32-8560 (1941-1942 CROPS)

Field group	Year	Age	Irrigations		T.S.A.M.	Avg. ac. per man-day	Avg. ac.in.* per ac. per rd.	Acres
			Number	Per mo.				
Koolau Mauka	1941	22.35	34.7	1.55	.644	9.93	5.98	624.00
	1942	23.00	31.1	1.35	.570	7.42	6.52	576.66
Koolau Makai	1941	21.08	32.3	1.48	.601	6.50	5.64	245.18
	1942	23.00	34.1	1.48	.545	5.94	6.76	323.30
Waianae	1941	20.39	28.3	1.39	.552	6.58	7.49	403.73
	1942	20.99	32.7	1.56	.530	7.05	8.44	152.43
Plantation	1941	21.51	32.3	1.50	.610	8.28	6.38	1322.91
	1942	22.71	32.2	1.42	.557	6.91	6.87	1052.39

* Acre-inch figures are based on gross water deliveries.

In Table II the irrigation applied to the 1941 and 1942 crops of 32-8560 is summarized. Among the field groups there has been a slight variation in number of rounds applied. The averages for the two crops are quite comparable. Although the Koolau groups have somewhat higher average number of rounds, there were fields in these areas that actually suffered from a lack of water in the two crops. Considering the limitations of water supply, it is felt that irrigation of the 32-8560 fields was as optimum as possible under operating conditions at the time.

Acre inches of water applied per round are, in general, somewhat below the plantation average. This is due to the fact that most of the 32-8560 fields have been located on the Koolau slope where soil conditions permit the use of less water per round. The Waianae group is low considering that acre inches are generally high in this area. New and more effective field layouts have contributed to more efficient use of water and labor.

With the spreading of 32-8560 there has been an increase in labor efficiency as associated with irrigation. The continued spread and improvement of Waialua flume and field layouts is primarily responsible for this increased man-day performance.

The following tabulation indicates the rate of improvement since 1938 when 32-8560 made its appearance:

IRRIGATION SUMMARY—MAN-DAY PERFORMANCE

Year	Average acres irrigated per man-day
1942.....	7.07
1941.....	6.46
1940.....	6.16
1939.....	4.62
1938.....	3.08

Thus far Waialua has been unable to associate any decrease in yield with the number of acres irrigated per man-day. However, this is a point that must be watched because maximum yields are the objective rather than high man-day performance achieved at the expense of thoroughness in irrigation. Quality irrigation must be maintained.

In the Koolau mauka group of fields, it appears that the reduced number of rounds of irrigation per month in the 1942 crop may have caused a reduction in sugar per acre month yields. Since there is not much differential in age and knowing that we did experience a water shortage in many of the fields harvested in the 1942 crop in that area, there is probably a significant effect of lack of irrigation on yields in this field group.

The Koolau makai group has a reduced yield in 1942 as compared with 1941 (T.S.A.M.); however, this is probably due more to age of crop and the particular fields harvested rather than to irrigation since a water shortage was not acute in this area.

The Waianae group of fields received adequate irrigation in both 1941 and 1942, although the intensity of irrigation was greater in 1942. Due to the small acreage of 32-8560 harvested in this field group in 1942, the effect of individual fields might be dominant and it is questioned whether the 1942 yield average in this area is truly representative. Irrigation is not a limiting factor in this area as related to water supply. In the poorly drained fields it is possible to overirrigate. It has been observed that 32-8560 responds readily to drainage. The Kawaihapai fields afford an excellent example of this.

32-8560 in ratoon crops is particularly sensitive to inadequate irrigation. In first ratoon fields where the grade of line, shape of line, length of line, flow control,* and soil condition have not been conducive to adequate penetration, 32-8560 is visibly affected. Sharp lines of variable growth have been noted. This is associated with fertilizer distribution, too; however, inadequate irrigation is a principal cause. The tendency of 32-8560 toward rapid rank growth where conditions are optimum undoubtedly causes this differential between good and poor growth areas to appear serious. However, it is felt that 32-8560 in the poor areas is still better than other varieties that have been grown in the same poor spots. Steps are being taken to minimize the range of variation caused by those factors mentioned above.

Ripening of 32-8560:

The so-called ripening or drying off of fields prior to harvest is a phase of cul-

* Flow control refers to the control of water going into individual furrows.

tural practice which is decidedly weak. The difficulty in predicting weather for even a few months in advance makes the scheduling of ripening a difficult problem. Fields at Waialua vary in their environmental factors (soil, drainage, rainfall, and temperature) that affect ripening and sound differential treatments are difficult to attain.

Ripening practice is felt to be an important part of the growing of 32-8560. For that reason considerable study has been made in an attempt to develop correlations with yields and juice quality that could be practically applied in terms of field practice.

On the basis of data available, we are unable to establish a positive relationship between the number of days water is off prior to harvest and the juice quality obtained at harvest. Due to the uncontrollable factor of rainfall, it was felt that perhaps the number of days the fields were below wilting point during the ripening period would permit a better correlation; however, here again no reliable trend was noted. It, of course, makes a difference as to when the days below wilting occur. If they should occur early in the ripening period and adequate moisture is available in the field at harvest, the probability would be that other than optimum juices would be obtained.

To check this point the number of days below wilting point directly prior to harvest was studied. Here the result was somewhat better. Although we are unable to state the exact number of days below wilting point that should be allowed to attain good juices (nor would we be able to control them if we did know), it is evident in fields where good juices have been obtained that the soil should go below the wilting point directly prior to harvest to obtain the best juices. The total days of ripening actually mean very little. The ripening time that really counts is the period directly prior to harvest. Since the uncertainty of rain makes it impossible to set up a precision control on ripening, the best that can be done in the way of intelligent field practice is to consider each field individually, weigh the time of harvest against the season, age of crop, soil moisture (such as drainage problems) and set a ripening period that will allow for optimum ripening—average weather conditions permitting.

The leaves of 32-8560 do not change in color during ripening to the same extent as H 109. Due to the dark green color that is characteristic of the variety, it appears to be evident that when 32-8560 looks dry—it is too dry for maximum yields. It appears that 32-8560 should not be brought to a stage of drying-off where it appears as yellow in color as H 109. We have had instances where a field was dark green in color for an April harvest. The drying-off directly prior to harvest appeared optimum for this time of harvest (30 days below wilting) and a 7.68 juice quality was obtained.

A phase of ripening that should be considered and developed is the treatment of the field prior to ripening. With a rank-growing variety such as 32-8560, there is the possibility of deterioration. In addition there is the danger of abruptly taking the water off of a crop when it is in a state of rapid growth. The conditioning of a field for ripening, particularly for the summer and fall harvest months is one that deserves consideration. A field that has had extended irrigation intervals for a 3- to 5-month period prior to the initiation of ripening would be hardened and would be in a better condition to withstand the rigorous treatment imposed on a field that is ripened during the hot summer months. This, in combination with shorter ripening during

this period, should be a step forward in ripening policy. This would also afford an opportunity to control the high rate of growth during the harvesting season. (Note Fig. 1 on Average Weekly Growth by growing seasons.) This would, as noted elsewhere in this report, bring the cane in better condition to harvest and would possibly improve the juice quality obtained.

32-8560 and Fertilization:

Under Waialua conditions fertilizer represents one of the more important factors available for the control of growth and sugar production. This is particularly true with 32-8560 for here is a variety that appears to be sensitive to excessive amounts as well as to inadequate amounts. This refers particularly to nitrogen fertilization. Over fertilization with nitrogen results in rapid rank growth, that is subject to poor condition and juice quality that is not optimum. Although yields obtained thus far indicate that fertilization has been adequate, it is felt that Waialua still has progress to make in finding both amounts and time of applications that will increase the effectiveness of 32-8560 fertilization practice. In general, the trend is toward reduced amounts of nitrogen in plant crops. Ratoons in some instances may need increased amounts.

Nitrogen: Nitrogen fertilization for 32-8560 was originally based on an approximate 200-210 pounds per acre for H 109 with a 20-pound per acre reduction for 32-8560. Due to the growth characteristics of 32-8560, this was felt to be a safe reduction. Experiments that were subsequently harvested indicated no significant gains in sugar over 150-160 pounds per acre. Nitrogen field practice has continued between 180-190 pounds per acre.

On the basis of observation made, thus far, it appears that Waialua may be somewhat high in nitrogen fertilization of 32-8560 plant crops. Where nitrogen is evenly applied in experiments, 160 pounds per acre appears to be sufficient. This would probably result in slightly lower tonnages; however, juice quality and economic sugar production would undoubtedly benefit.

Although Waialua has no experimental evidence to substantiate this point, it does appear that the ratoon crops of 32-8560 do not show the growth or color that is characteristic of plant crops of the variety. On the basis of observation of fields and yields, it appears that it may be profitable to reduce the nitrogen amounts from 180-190 to 160-170 pounds per acre in plant crops of 32-8560 (depending on age and cropping). The 20 pounds saved on the plant crops could probably be applied to advantage on certain ratoon fields where there is fragmentary evidence that the ratoon yields may decrease due to a shortage of nitrogen. Due to the difficulties found in the obtaining of even fertilizer distribution within a field, it is sometimes felt that the importance of a 20-pound or even a 40-pound application of nitrogen can be over-emphasized in research studies. Field practice in many instances is confronted with variations in plant food distribution that exceed the differentials mentioned. There is as much a need for even fertilizer distribution as there is for the control of fertilizer amounts when these amounts are within 20 to 40 pounds of the optimum.

Mechanical or hand methods of nitrogen application should be used if possible. All ammonium phosphate applications should be applied by hand or mechanical means, not by water. Fertilizer in this form does not go into solution readily and water applications are subject to considerable variations in actual distribution. Im-

provement of soil tilth by subsoiling in ratoons should be a step toward better irrigation in ratoons and as a result more satisfactory plant food distribution where fertilizer is applied by water. 32-8560 is as responsive if not more so to adequate amounts of plant food and water as the older varieties.

Nitrogen applications in the past three crops of 32-8560 have been as follows:

TABLE III
32-8560—SUMMARY OF NITROGEN FERTILIZATION BY SEASON AND FIELD
GROUPS 1940-41-42 CROPS
(Pounds Per Acre)

Field group	1940			1941			1942		
	First season	Second season	Total	First season	Second season	Total	First season	Second season	Total
Koolau Mauka ...	168	...	168	84	105	189	76	117	193
Koolau Makai	111	79	190	34	156	190	127	60	187
Waianae	25	172	197	91	88	179

There has been very little variation in the general nitrogen treatment of 32-8560 except as developed by differences in cropping. In general the amounts have been held about 20 pounds per acre less than comparable H 109 fields.

Following is a rule-of-thumb basis of determining the approximate amounts of nitrogen to be applied during the first and second season of crops at Waialua based on the time of start and the time of harvest:

TABLE IV
32-8560—APPROXIMATE AMOUNTS OF NITROGEN BY MONTH OF START
(Pounds Per Acre)

Month of start	First season	Second season	Total
January, February, March	147	42	189
April, May, June	126	63	189
July, August, September	84	105	189
October, November, December	63	126	189

Until such a time as we are able to use effectively the plant as an index of its plant food requirements, a basis as given above must be followed in some form. Table IV as given is approximate, as each field received individual attention as to its fertilizer schedule. The general pattern as indicated is based largely on past experience and field experiments. We are somewhat above the maximum indicated by experiments for plant crops of 32-8560. Realizing the variations introduced by water applications of fertilizer, this margin over the maximum as indicated by experiments, has constituted a safety factor.

Phosphate: The use of phosphate in 32-8560 fields has followed the practice used for H 109 and other varieties. In general approximately 100 pounds of P_2O_5 is applied per acre; however, there are instances where soil analyses have indicated the P_2O_5 content to be low and increased amounts have been applied to the field in question. A large amount of soil data pertaining to P_2O_5 has been accumulated and it serves as a guide in determining amounts to be applied. Thus far, in the growing of 32-8560, we have had no serious indications of P_2O_5 shortages. There are pali areas of poor soils where spot fertilization is necessary. Phosphates to be most effective should be applied either by hand or mechanically, and preferably below the surface, to achieve the best results. We do this in our planting operation where super phos-

phate is applied by machine and is rather well covered in the seed-covering process. In plant crops phosphates are applied at time of planting. In ratoon crops phosphate is usually applied a month to six weeks after the crop is started.

TABLE V

32-8560—SUMMARY OF P_2O_5 FERTILIZATION FIELD GROUPS 1940-41-42 CROPS
(Pounds Per Acre)

Field Group	1940	1941	1942
Koolau Mauka	105	120	100
Koolau Makai	102	88	103
Waianae	87	104

Potash: Potash fertilization in relation to 32-8560 has conformed very closely with general plantation practice. This amounts to about 122 pounds per acre of K_2O per crop. In some areas where soil analyses indicate this element to be low, the amount is raised to 183 pounds per acre. In many of the makai fields where the K_2O level is quite high, the amounts are adjusted downwards accordingly. In general all K_2O is applied with the second application of nitrogen. That is, in plant crops, K_2O would be applied when the cane is 4-6 months of age and in ratoons when the cane is 3-5 months of age. Although not always true, this practice tends to bring a field into its first winter with ample K_2O to draw upon and with a moderate supply of nitrogen. It has been suggested by the Experiment Station that the K_2O/N balance in the winter months should be in favor of K_2O and low on the nitrogen side. Such a practice has not been definitely confirmed under Hawaiian conditions as yet. Our practice approaches the suggested one in many cases.

K_2O is applied in the form of muriate of potash. Applications in the past few years have been made principally by water. Muriate of potash dissolves readily and can be applied by water effectively as long as irrigation practice is good.

TABLE VI

32-8560—SUMMARY OF K_2O FERTILIZATION BY FIELD GROUPS
FOR 1940-41-42 CROPS
(Pounds Per Acre)

Field Groups	1940	1941	1942
Koolau Mauka	122	169	134
Koolau Makai	110	123	138
Waianae	123	122

32-8560 and Growth Rates:

On the basis of observation and yield data collected, 32-8560 has proved itself to be a strong grower. Fig. 1 on Average Weekly Growth by starting, growing, and harvesting year indicates the growth picture of 32-8560 to be much the same as H 109 except that the rate for any given month is substantially greater with 32-8560. This has been assumed to be true; however, this is the first evidence that has been developed with Waialua data to substantiate our observations.

These growth data are of interest and if interpreted properly can be of assistance in planning cropping, fertilization, irrigation, and perhaps planting with the objective of effectively using time and growth to realize the maximum production of sugar with 32-8560.

AVERAGE WEEKLY GROWTH
32-8560 AND H 109
40-41-42 CROPS

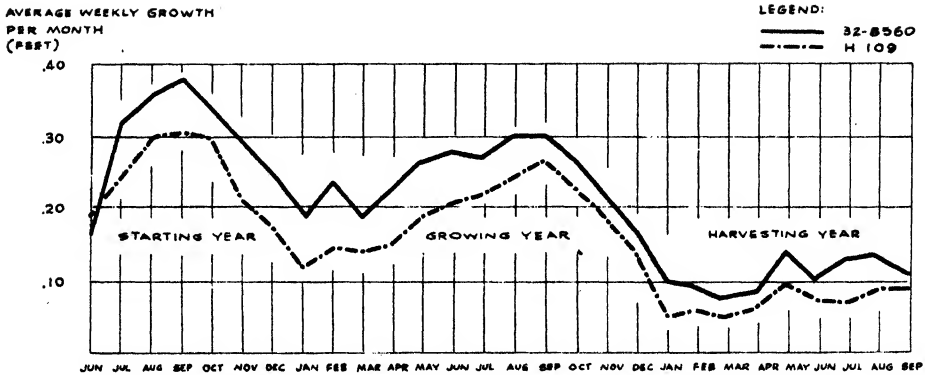


Fig. 1

For example, effective cropping makes use of various combinations of growing time, growth and production factors to obtain the greatest sugar yields possible. The trends shown in Fig. 1 can be of definite assistance by indicating the periods when the most growth can be anticipated. If a field of 32-8560 is started in June and scheduled for harvest 22 months later in April, it is apparent that the growth in the second January, February, and March may not be sufficient to justify the extra time given to the particular field. It might be more profitable from the standpoint of growing time to harvest in January or February. A compensating factor for the loss of growth could be an expected improvement in juice quality. The growth of 32-8560 from May to September in the harvesting year indicates that control is needed to reduce the growth rate for fields harvested during this period if the sucker population is to be minimized and juice quality improved. Various interpretations can be made with data of this type. The curves shown in Fig. 1 are subject to change and modification as more data are collected. They reflect the trends in 32-8560 growth as compared with H 109 under a specific combination of growth and cropping conditions.

Interpreting these growth data in terms of fertilization, it appears that first-season applications of nitrogen should be applied early in the crop to permit the full recovery of growth that is possible in the starting year. Losses in growth due to late and poorly timed applications could be considerable. Second-season applications at Waialua have a better chance for rapid absorption if applied in March and April rather than in January and February of the growing year.

These data may also have a bearing on irrigation practices. During the starting year and growing year, irrigation should be maintained at an optimum rate with the possible exception of the first winter season when extended intervals would be in order due to the reduced rate of growth. It appears possible in the harvesting year to extend intervals. Growth during this period is low and controlled irrigation would result in labor economics as well as increasing the probability of putting fields in better condition for harvest. Juice quality would undoubtedly be improved.

Although 32-8560 does better with late planting than other varieties we have

spread, experience would indicate that late planting should be avoided when possible. More sugar per unit of growing time can be obtained by planting in months that make possible the greatest recovery of growth.

Returning for a moment to cropping, these data represent average growth. By a study of individual fields, the growth rates would indicate to a considerable extent, in conjunction with yields, what trends future cropping should follow. I have attempted here to suggest various ways in which data of this type can be of assistance in guiding field practice.

Yields of 32-8560:

Soil Type and Elevation as Factors Affecting Yield: In a study of 32-8560 that was made last year on the basis of yields to that time, three large field groups were set up. The purpose in doing this was to determine, if possible, the effect of elevation and soil type on the yields of this variety. The field groups are identified as follows:

GROUPING OF FIELDS

Koolau Mauka (Kemoo, Helemano, Opaaula, Kawailoa
and Waimea Divisions above 300 feet)
Koolau Makai (Kemoo, Helemano, Opaaula, Kawailoa
Divisions below 300 feet)
Waianae (Ranch and Kawaihapai Divisions)

Koolau refers primarily to the red residual soils that occur in fields on the Koolau slope and which comprise practically all the soil in that area. In the fields constituting the Waianae soils, the principal types vary from alluvial to sedimentary soils and are made up of silty clay loams, sands, and rocky alluvials. The Waianae group includes elevation from sea level to 300 feet. A small area of brown residual soils is present in this latter group.

In last year's study, trends in yields were observed which were felt to be associated with elevation and soil type. The same field groups are being used in this report.

Table VII indicates that the five-crop mean yields of the fields harvested in 1942 are somewhat higher than the fields harvested in 1941. Per cent gains are thus greater for the 1941 32-8560 crop in the Koolau mauka field groups. The factor of water shortage had some effect on the 1942 yield because one would expect the 1942 fields to give higher yields based on the past performance of the fields involved. Since 1940, 1941, and early 1942 were quite dry, there may have been an accumulative effect of deficient rainfall on yields. The age differential between the two crops is not sufficient to cause the difference in yield. Juice quality was not as good as in previous crops but not bad considering the tonnage of cane obtained. This mauka group of fields was formerly the poor yielders. 32-8560 has completely changed the picture and this group of fields now represents the best producing areas at Waialua.

The five-crop mean yields of the Koolau makai fields harvested in 1941 and 1942 are quite comparable. The per cent gains of fields in the 1942 crop compare favorably with the 1941 fields in T.C.A., T.S.A., and T.C.A.M. 1941 32-8560 fields were better in T.C./T.S. and T.S.A.M. Age of crop in 1941 undoubtedly caused a more favorable sugar per acre month figure for that year. Cane and sugar per acre month figures in terms of per cent gain over previous yields are not quite as satisfactory as those in the Koolau mauka group.

Table VII shows quite a difference in the prior mean yields of the Waianae fields harvested in 1941 as compared with 1942. This naturally gives the 32-8560 harvested in 1941 a considerable advantage in per cent gains as compared with the 1942 crop. Due to the higher yields of previous crops in this group, 32-8560 does not show the large gains in yields that are indicated in the Koolau mauka and makai groups. Still, the gains are sufficient to justify the planting of 32-8560 in makai areas. At the same time we are continuing to search for a lowland variety that is even better suited than 32-8560 to the environmental conditions in this area. Due to the small number of acres harvested in 1942, the individual fields undoubtedly had a dominant effect on yields and the averages as given are probably not representative of the entire area.

As was indicated in last year's study, the Koolau mauka fields above 300 feet elevation showed the greatest per cent gains over previous yields in the same fields, as well as having the highest sugar per acre month values for 32-8560 as compared with the other two groups. The Waianae group which constituted the most productive fields has now exchanged its rating with 32-8560 in the mauka fields. This indicates that H 109 was much better adapted to the lowlands and that in the mauka areas that variety was out of its element. For this reason the gains for 32-8560 when expressed in per cent are naturally higher in the mauka areas. However, actual mean T.C.A., T.S.A., T.C.A.M. or T.S.A.M. as shown in Table X substantiate the superiority of the Koolau fields in growing 32-8560. Considering the average yields of these field groups, there appears to be an effect of soil and elevation on yields. Since these yield data are made up principally of plant crops, which are usually better than ratoons at Waialua, it will be particularly interesting to compare yields after sufficient ratoons have been harvested to make possible a better comparison with past yields. All 32-8560 fields included in this study were mechanically harvested. Previous yields in the same fields were handled principally by the hand-cut and hand-load or machine-load method.

Table IX summarizes the per cent gains and losses. The Koolau mauka group is superior as a location for growing 32-8560 on the basis of per cent gains over previous yields in the same fields.

Table X indicates that the superiority of yields in the Koolau mauka group is not only expressed in per cent gains over previous yields but also in actual comparison of yields. In both 1941 and 1942 there was a downward trend in sugar per acre month figures from mauka to makai. In 1941 cane per acre month did not vary materially in the three groups. In 1942 there was slightly more variation; however, other than for the fact that cane production was lowest in the makai group, no particular trend is in evidence. The plantation summary brings out the decrease in yields obtained in the 1942 32-8560 fields as compared with the 1941 yields.

The data in Table X point out that the greatest gains are to be made with 32-8560 in our mauka and makai fields on the Koolau slope. Attention is also drawn to the fact that 32-8560 in the Waianae group of fields requires careful study and care in its culture to obtain the most possible from a variety that is not too well adapted to these conditions, even though it does give evidence of being better than previous varieties grown in this same area.

Record Yields of 32-8560: 32-8560 has set field records of some sort in practically every field in which it has been planted. Thus far, Opaepa 5A holds the pro-

duction record on cane having produced 136.17 tons of cane per acre. This same field holds the record on tons sugar per acre having yielded 16.20 tons of sugar per acre. The best juice quality to be obtained in a 32-8560 field is from Kemoo 2B where a quality of 6.71 was obtained. Waimea 5 in a short plant crop harvested in 1940, holds the record on tons cane per acre month. The figure here was 5.79 tons of cane produced per acre month. Helemano 11 which was harvested in April of 1941 at an age of 20.97 months, set a record of .708 ton sugar per acre month that still stands as the record yield of 32-8560 at Waialua, as well as the all time plantation record for a field containing 179.00 acres.

The magnitude of these record yields indicates to some extent the possibilities in growing this variety. There is a real challenge presented in that the maximum economic yield of sugar should be the goal in each field.

TABLE VIII

32-8560—SUMMARY PLANTATION YIELDS COMPARING 32-8560 YIELDS
IN 1941 AND 1942 COMBINED FIELD GROUPS

Yields with age and area data	1941 32-8560 yields	5-Crop average yield	% +or—	1942 32-8560 yields	5-Crop average yield	% +or—
T.C.A.	101.37	75.76	+34	101.48	79.82	+27
T.S.A.	13.12	10.42	+26	12.63	10.74	+18
T.C./T.S.	7.72	7.27	— 6	8.03	7.44	— 8
T.C.A.M.	4.71	3.24	+45	4.47	3.44	+30
T.S.A.M.610	.446	+37	.556	.463	+20
Age	21.51	23.38	—	22.71	23.20	—
Area	1,322.91	6,349.14	—	1,052.39	6,764.76	—

TABLE IX

32-8560—SUMMARY OF PER CENT GAINS AND LOSSES

	Koolau—Mauka		Koolau—Makai		Waianae		Plantation	
	1941	1942	1941	1942	1941	1942	1941	1942
T.C.A.	42	30	26	35	23	— 2	34	27
T.S.A.	38	23	23	22	9	— 7	26	18
T.C./T.S.	— 3	— 6	— 3	—13	—12	— 4	— 6	— 8
T.C./A.M.	50	32	36	31	36	10	45	30
T.S.A.M.	46	25	33	18	21	5	37	20

TABLE X

32-8560—SUMMARY OF T.C.A.M. AND T.S.A.M. YIELDS—1940-1941-1942

Crop	Koolau Mauka		Koolau Makai		Waianae		Plantation		Total area
	T.C.A.M.	T.S.A.M.	T.C.A.M.	T.S.A.M.	T.C.A.M.	T.S.A.M.	T.C.A.M.	T.S.A.M.	
1940.....	5.24	.561	4.99	.587			5.19	.566	292.07
1941.....	4.72	.644	4.73	.601	4.69	.553	4.71	.610	1322.91
1942.....	4.45	.570	4.63	.545	4.14	.525	4.46	.556	1052.39
Ttl. average	4.67	.606	4.72	.577	4.45	.541	4.66	.584	2667.37

Some Trends of Factors Affecting Cropping:

The proper cropping of a variety is dependent upon a number of factors. One thing certain is that optimum cropping is usually available for only a portion of the total fields in a crop. However, the possibility of cropping all fields to advantage and making the best out of each particular season through which a field passes in reaching maturity is always present.

It is essential to know something about the variety in terms of the proper time to start a crop, the range of age when maximum yields can be expected, and the month of harvest, which in combination with optimum age will give the most effective yield. Although all factors affecting growth are cropping factors, the phases discussed here refer principally to per acre month yields as related to time of start, age at harvest, time of harvest, and juice quality as related to month of harvest, as well as age.

Limitations of operations make it impossible to start and harvest all fields at the most optimum time for the best quality of juice and maximum sugar yields. Effective cropping requires continual analysis so that 32-8560 can be made to produce the maximum economic yield of sugar consistent with the time of start, time of harvest, and age of crop involved.

The data that Waialua have available for a study of the cropping factors of 32-8560 are limited to forty-five fields. These have been harvested over the past

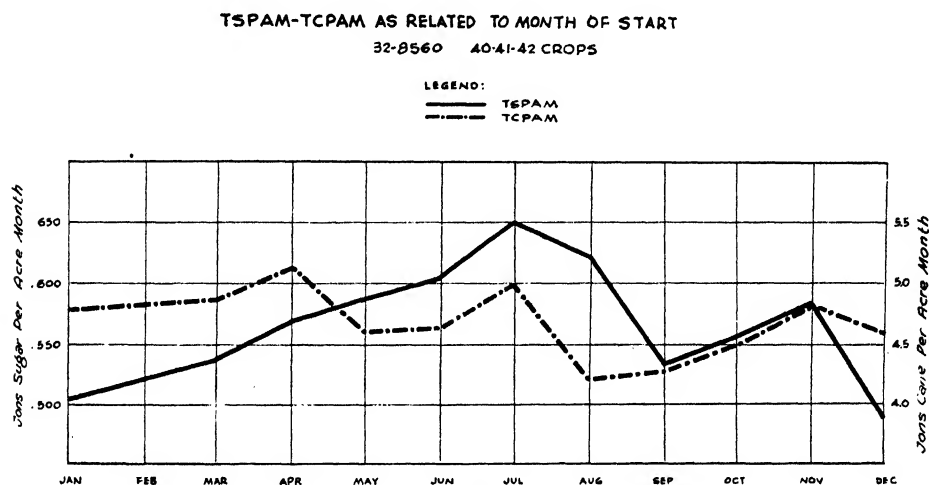


Fig. 2

three years. The greater proportions of these represent plant crops. There are insufficient data available for a study of ratoons as related to plant crop yields. For the purpose of this report, the plant and ratoon crops have been combined. Additional data will undoubtedly modify the trends shown in the following graphs.

Time of Start: Fig. 2 indicates the trend of 32-8560 yields as related to the month of start. It is shown on the basis of available information that April, May, June, July, and August have been the best months in which to start 32-8560 crops. The peak months occur in June, July, and August. It is surprising that the favorable months of start extend so far into the summer months. This is due, perhaps, to the lack of a substantial number of fields started in the early months of the year. There is a possibility that due to 32-8560's tendency toward rank growth, a midsummer start allows the crop to start well and at the same time puts two winter seasons and a growing summer in the proper place to exercise a favorable control on cane and sugar production. A similar control can also be afforded through fertilizer practice and irrigation control, at least in part. 32-8560 is able to sustain good growth

at lower temperatures than H 109 and the winter months do not constitute such a critical period of growth reduction. This can be noted in the graph on Average Weekly Growth (Fig. 1).

TSPAM-TCPAM AS RELATED TO AGE AT HARVEST

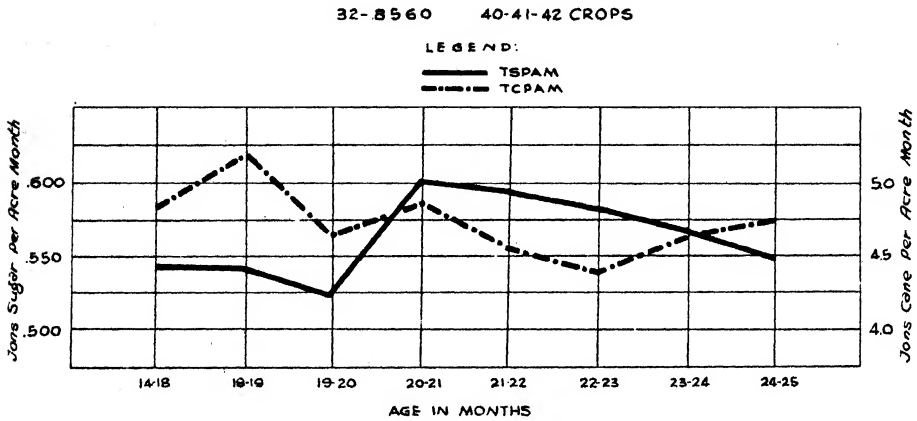


Fig. 3

Age to Harvest: Fig. 3 indicates the trends as to the proper age to harvest 32-8560 to obtain the best T.S.A.M. values.

This graph shows that the best age to crop 32-8560 is between 20 and 24 months. In this range of age, the most satisfactory sugar per acre month production came between the ages of 20 and 21 months. However, the manner in which sugar per acre month production is maintained up to 24 months points toward a 20-24-month cropping age as giving the best yields.

T.C.A.M. production is highest in the lower age groups and decreases gradually

TC/TS AS RELATED TO AGE AT HARVEST

32-8560
40-41-42 CROPS

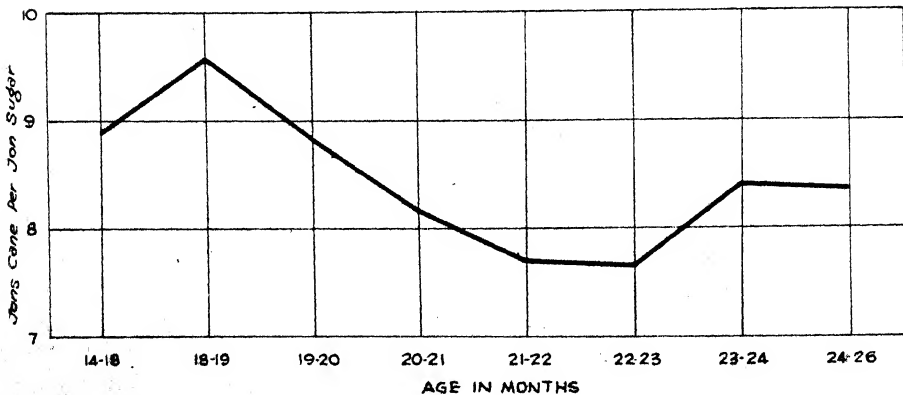


Fig. 4

with increased age, although beyond 24 months there is an indication of a rise in T.C.A.M. We have evidence that 32-8560 will carry over in good condition beyond 24 months if necessary; however, due to the decreasing rate of sugar per acre month production, it is felt that excessive age should be avoided if at all possible.

T.C./T.S. and Age at Harvest: On the basis of available data, juice quality appears to improve with age up to 23 months. A graph of this sort does not give the complete picture because the cropping, especially the month of harvest, has a dominant effect on juices obtained, other factors being equal. T.C./T.S. as related to age is set forth in Fig. 4.

TC/TS BY MONTH OF HARVEST

32-8560
40-41-42 CROPS

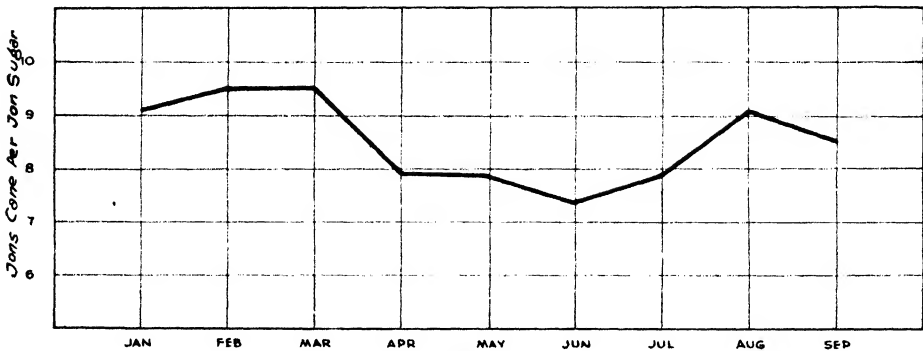


Fig. 5

T.C./T.S. by Month of Harvest: Fig. 5 sets forth trends in juice quality of 32-8560 as associated with month of harvest.

TSPAM - TCPAM AS RELATED TO MONTH OF HARVEST

32-8560
40-41-42 CROPS

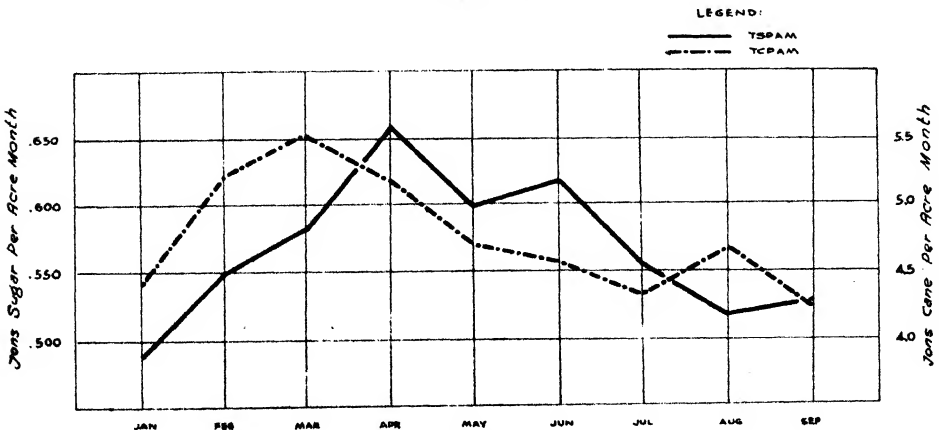


Fig. 6

Starting with March, juice quality steadily improves through June. In July there is a slight decrease in quality and in August, juices are somewhat poorer. The best juice months are April, May, June, and July.

Here again it is necessary to look beyond this graph to the others presented here. T.C./T.S. as related to age is a factor. Age at harvest is also a factor. In other words the combination of age and time of harvest influences considerably the quality of juice obtained.

Month of Harvest: Fig. 6 shows T.C.A.M. and T.S.A.M. as related to month of harvest for the variety 32-8560.

February, March, April, May, June, and July appear to be the best months in which to harvest for maximum sugar per acre month production. April, May, and June are the peak months. This trend is consistent with past experience.

Comments on Cropping:

It appears that 32-8560 does best when harvested between the ages of 20 and 24 months. Per acre month production decreases with increased age so crops should preferably be between 20 and 22 months of age; however, 32-8560 does well enough at 22-24 months to justify amply crops of that length. Since the best economic returns are not always associated with maximum sugar per acre month production, the carrying ability of 32-8560 in crops over 22 months of age is of definite interest.

On the basis of observations and yields, crops harvested late in the year (August, September, and October at Waialua) should be in the age range of 22 to 24 months for best results. This is a period of declining juice quality and 32-8560 which is a little older and with fewer immature stalks is better suited for harvest at this time than is a 20- to 22-month crop. Still younger cane harvested late in the season is even more dangerous.

If it is necessary to take short crops, it is felt that 14 to 16 months cane is more satisfactory than 16 to 19 months cane, particularly if the fields are to be harvested late. In the 14 to 16 months group, the suckers have not had time to develop to the point where juice quality is adversely affected to a serious extent. Fields 16 to 19 months of age harvested late at Waialua have given relatively poor yields compared with similarly aged fields harvested during the period from April to June. In general it is felt the short crops of 32-8560 should be avoided whenever possible.

32-8560—Diseases and Pests:

In addition to being a versatile variety in relation to soils, elevations and climate, 32-8560 is outstanding in its resistance to plant diseases and insect pests.

Plant Diseases: At Waialua no major plant disease has made a serious threat to the variety.

32-8560 has proved to be resistant to eyespot disease. This represents a real stride forward in disease control at Waialua. The use of a resistant variety has practically eliminated the presence of a disease that a few years ago was causing real financial loss to Waialua. This is an outstanding example of disease control by the use of a resistant variety.

Brown stripe has been observed, particularly in the mauka areas during the winter months when low temperatures and rainy weather dominate the environment. The extent of brown stripe during this period of the year has not been considered serious. 32-8560 can be considered rather tolerant to this disease.

Chlorotic streak, a potential pathological danger, has been observed in low poorly drained areas. Recently we have noted the disease in mauka areas where seed was taken from low poorly drained fields. As a precautionary measure we are trying to avoid the cutting of seed from such fields.

Due to the rank growth characteristics of the variety, a few scattered cases of knife-cut have been noted. Proliferation in ratoon stools has also been observed. Major plant diseases have been conspicuous by their absence and thus far have offered no problem in the growing of this variety.

Pests: Insect pests have not found 32-8560 to their liking. At least conditions have been such that damage has been minor for the period in which 32-8560 has been grown at Waialua.

Rat damage is, of course, chronic but as long as rat control measures are continued, there will be no threat from this source.

Armyworms have appeared on 32-8560; however, due to the prevalence of parasites in the district, we have had no epidemics of serious proportions. No other insect pests have caused damage on 32-8560 sufficient to recognize them as serious pests.

Milling Characteristics:

Among the newer varieties, 32-8560 resembles H 109 more closely in milling than any of the others. 32-8560 is harder on the knives. Tests have shown that the knife motors require more power when handling this variety. This is probably due to the long stringy fiber. Boiling house characteristics are about the same as H 109.

In the fireroom there is again a striking similarity between 32-8560 and H 109. Bagasse samples from this variety are usually lower in both moisture and pol than are most other varieties. It is felt that this may be, in part, due to the fact that practically all of the 32-8560 harvested through 1942 has been plant cane.

32-8560 has been observed to deteriorate more rapidly than H 109 after burning and harvesting. This has also been observed on other plantations. There is the feeling that this is largely due to the tendency of 32-8560 to split and tear rather than to have a clean break. Mechanical harvesting develops much cane that is in this condition. After rains, if the cane is slow in getting to the mill, there is usually considerable sour cane. To minimize the amount of deterioration, a close control on sizes of burns is needed. For that reason there should be close cooperation between the harvesting superintendent and the mill.

Harvesting Characteristics of 32-8560:

From a harvesting point of view, 32-8560 is more like H 109 than any other variety we have grown. H 109 in the harvesting field as in the mill is looked upon as the best cane to handle. The fact that 32-8560 comes very close to H 109 is fortunate.

Grab-harvesting has found the stools of 32-8560 to be strong, and they do not pull out excessively. Like H 109, 32-8560 will either break off at the surface of the ground or will leave a 3- to 5-foot stalk which can be readily handled by the ground crew. It is vastly superior to 31-2510, H 8965, 31-2347, P.O.J. 2878, 27-8101, 31-1389, 31-2806, and others that have come through the variety parade of the past years.

Due to the rank growth that develops with 32-8560, the quality of burns obtained

are usually not as good as with H 109. The persistent green tops generally never dry up and burn as well as does H 109. This results in a somewhat higher per cent trash with this variety.

With the present form of harvesting being used, 32-8560 seems well adapted to grab-harvesting due to its heavy tonnages.

The principal fact of interest is that this variety has no serious handicaps that affect its harvesting. It has been generally observed, due to its growth habit, that it can withstand more rigorous treatment than can, for example, H 109. With our present method of harvesting, this is of real importance.

A Summary of 32-8560 at Waialua:

The spreading of 32-8560 started in 1938 and increased in area with each succeeding year. Sixty-nine per cent of the present cultivated area is now planted with this variety.

Germination and subsequent stands of the variety have been good. In general, replant has been light. The variety offers no problems in cultivation at Waialua and indications are that ratoons will be cheaper than plant cane to cultivate.

Good irrigation is required to obtain maximum yields with 32-8560. There is evidence that ratoons will require careful irrigation to obtain comparable yields with plant cane.

Ripening is still an uncertain factor with 32-8560. Much care and study is needed to ripen 32-8560 effectively within the range of controllable factors. Further studies are being made.

Fertilization has been close to optimum. Recommendations for a nitrogen reduction in plant 32-8560 are made. It is suggested that this can be applied where needed in ratoon fields of the variety. Phosphate and potash practice appears to be satisfactory.

The growth rates of 32-8560 are shown to be considerably better than H 109. The interpretation of these growth data is of real importance in relation to cropping and cultural practices.

It is shown that 32-8560 gives its best yields in the mauka areas above 300 feet elevation. This refers to both actual yields and per cent gains over previous yields in the same fields. The mauka fields have displaced the makai areas for the time being, at least, as the most effective sugar-producing area at Waialua.

Lack of sufficient data makes a study of ratoon yields difficult at present. Suggestions for improved cultural practice in ratoons have been made.

Several graphs pertaining to factors affecting the cropping of 32-8560 are presented. Although based on averages, the data point out several trends which are of importance in effective cropping.

32-8560 has been found to be resistant to plant diseases and pests. Milling and harvesting characteristics of the variety are touched on briefly.

Acknowledgments:

Appreciation is extended to A. J. Mangelsdorf and the Experiment Station staff for many helpful suggestions and criticisms. Much help and assistance was given by the Production staff at Waialua Agricultural Company, Limited. The Waialua

staff has contributed a great deal to the development of 32-8560 at Waialua and thus to this report. James Hagio and others of the Agricultural-Irrigation Department helped considerably in the assembling of data and associated graphs. John H. Midkiff made the data available for publication.

Sugar Prices

96° CENTRIFUGALS FOR THE PERIOD
MARCH 15, 1943, TO JUNE 14, 1943

Date	Per pound	Per ton	Remarks
Mar. 15, 1943-June 14, 1943.....	3.74c	\$74.80	Philippines

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Yield Variations With Special Reference to Border Effects in Field Tests

By R. J. BORDEN

Largely due to observations made during the early growth of sugar cane upon the outer rows of adjacent, differently treated plots in field tests, opinions have differed concerning the extent of the influence on final yields which could be due to border effect. Hence from two recently harvested nitrogen tests at Waipio, evidence was sought to prove the existence of such effects. The results lead us to the following conclusions: (a) When the plan of the experiment is such that the treatment differentials for adjacent plots are apt to cause large differences in final yields, a rather definite border effect will be found, but (b) when the treatments on adjacent plots are not greatly different and the expected yield differences are apt to be quite small, then little or no border effect can be expected.

It is not an uncommon occurrence, when making observations of the growth of sugar cane during its early development while the stalks are still erect, to find a lack of uniformity and to see some rather definite growth differences. Unfortunately we have little proof of what actually happens to these early growth differences after the cane goes down and so we do not know whether or not they are carried right through to harvest and actually affect the final yields. Similarly and perhaps just as often, our observations reveal a remarkable degree of growth uniformity during these first 6 or 8 months of development but results at harvest indicate that this early uniformity is not always carried through the crop. Thus it is quite apparent that different sorts of growth influences become operative during the second half of the growth period which greatly modify the cane growth made earlier.

There is some evidence from field tests that early visual differences in growth may not have as great an influence on final yields as we are sometimes inclined to imagine. For instance, on most of our older cane lands when phosphate fertilizers are now supplied for a sugar cane crop at time of planting, a pronounced increase is generally seen over the check plots in the early growth and tillering, and obser-

uations of this initial stimulus frequently give rise to unduly optimistic estimates of final cane yields. However, as the crop develops, the growth differences between the phosphate-fertilized cane and the cane which has received no phosphate are found to gradually even up, and when the crop is finally harvested no significant differences in yield are found. In contrast, we have such observations as were made in Waipio Experiment 110 where there were no observable differences in cane growth for the first 5½ months between the "no-nitrogen" and "nitrogen-treated" plots, and yet at harvest 15 months later a very substantial difference in cane yield favored the latter.

The observation of early growth differences in adjacent but differently treated plots in field tests has sometimes led to an unwarranted interpretation, and has even cast some doubt on the validity of final yield figures submitted after harvest. Our initially formed opinion is apt to be biased by what we see early, and because we do not see the changes which take place later, our first judgment persists. It is our opinion that our observations cannot be depended upon to show a high correlation with final yields, because growth influences, the effects of which are not easily recognized after the cane is recumbent, can cause great changes in factors which contribute to final cane yields.

If the preceding comments concerning early observations of cane growth are admitted, then it is easy to agree that "border effect" has been seen in some of our field experiments. But before we are willing to admit that such observed "border effect" has greatly influenced yield comparisons and led to a faulty interpretation of the results, we shall need better proof.

We shall define "border effect" in field tests as that difference in cane yield which results when one (or more) of the outside or border rows in a plot receives an advantage or a disadvantage in some growth factor which is not received by an inside row. Such factors as differences in nutrients, water, sunlight, and exposure to wind are apt to be these chief causes.

Since nitrogen tests are so important in our plan of research, we have sought our evidence from 2 nitrogen tests recently conducted at Waipio. In Experiment No. 110, a heavy nitrogen application (220 pounds per acre) was compared with no nitrogen in several groups of adjacent plots. This is probably a larger nitrogen differential than we would commonly use in an "amounts-of-nitrogen" experiment but it was planned this way in order to get a better idea of the maximum extent of any border effect which might be found.

Observations made at 3½ months showed no evidence of a difference in growth between the two treatments and not until 5½ months was it possible to distinguish any differences, and then it was chiefly a leaf-color difference which distinguished the treatments. Furthermore it is very doubtful that a border effect was actually observed while this cane was still erect.

When it was time to harvest the crop adequate supervision* was supplied to see that the cane was cut and weighed separately from each row in each plot. Since the rows were of variable length all row yields were subsequently calculated to a tons-per-acre basis and our studies have been made from these T.C.A. figures.

The yields of the individual rows which were harvested are graphically shown in Fig. 1. This test occupied 5 blocks (of 2 plots per block) in 4 separated areas

* By Messrs. Smith, Yamasaki, Ching, and McCall.

WAIPIO EXPT. 110AN - VARIATIONS IN YIELDS OF ADJACENT ROWS

YIELDS FROM EACH ROW AS TCA

LEGEND:

- D PLOTS @ 220 LBS. N/ACRE
- - -○- - X PLOTS, - NO NITROGEN

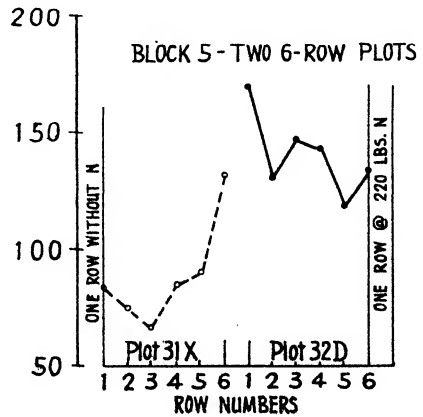
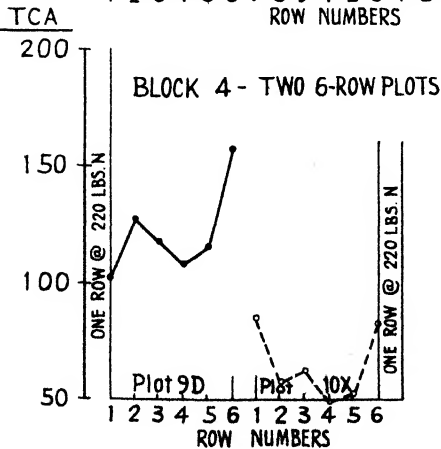
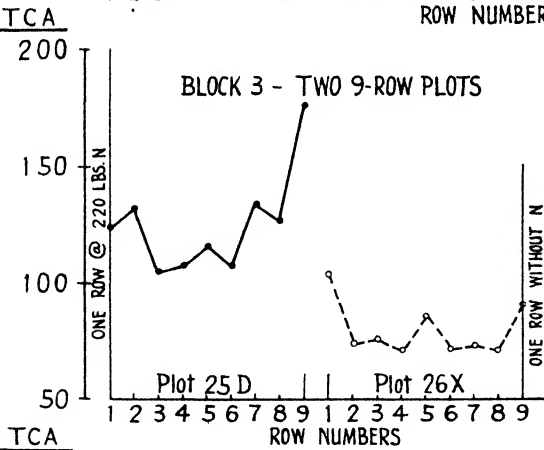
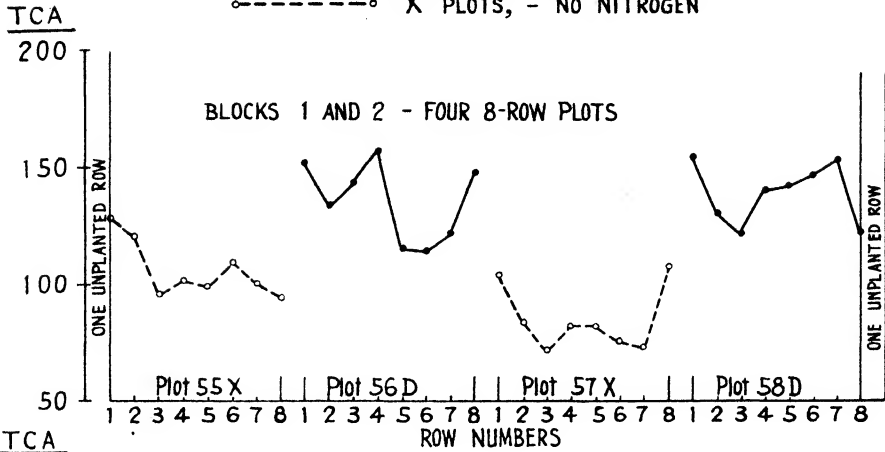


Fig. 1

in Field L. In blocks 1 and 2 there were 8 rows in each plot; block 3 had two 9-row plots, and plots in blocks 4 and 5 had only 6 rows each.

An inspection of Fig. 1 shows some rather clear-cut evidence of border effect as judged by the actual cane yields harvested and, as will be seen shortly, a statistical analysis of the yields from the border rows tends to confirm this evidence. Except for row 8 in plot 55 X, the border rows in the other plots with Treatment X (no nitrogen) appear to have been increased through their intimate association with cane in an adjacent row which had received nitrogen fertilizer; we assume that they "stole" some of the nitrogen. At the same time border rows in the "D" plots which received nitrogen also seem to have benefited by being next to cane which received none; apparently they were not adversely affected by their loss of nitrogen to their adjacent "no-nitrogen" cane and were benefited by the extra sunlight made available when growth of the stalks in the adjacent "no-nitrogen" row failed to keep up with them.

To support the evidence in Fig. 1 we have the following statistical analysis. We have made use of the yields from all rows except the border rows to determine the extent of the expected or chance variation which is probably not a border effect within this test area. Thus we find that after deducting the treatment effects and the block effects from the total variation for the 54 inside rows in this test, we have a standard deviation of 12.4 tons. With this figure for the chance error of a single row as our guide, we can assume that the individual row error which falls within an amount of " t "* times this standard deviation is quite likely an expected error for this area or population of cane, and therefore cannot be safely attributed to any specific border effect. If, however, the individual error for a border row is greater than the expected error (24.9 tons in this case), we are probably right in assuming that it has been influenced by some factor other than chance, and so we can more safely assume that a definite border effect has been measured.

To find the amount of individual error in the yield from each of the border rows, we first assessed the difference between each border row yield and the general mean yield of all 54 inside rows, and from this difference we then subtracted the variation due in each case to the specific treatment of the border row and also to that variation which is most likely due to its position in a specific block. This was done by use of the following formula:†

$\Sigma e = \Sigma [(x - \bar{x}) - (x_t - \bar{x}) - (x_b - \bar{x})]$; which when simplified becomes $e = x - x_t - x_b + \bar{x}$, where e = individual row error; x = yield of border row; \bar{x} = mean yield from inside rows; x_t = treatment average; and x_b = block average.

This calculation and its results are shown in Table I, being broken down into 6 groupings to allow for slight differences in their border row exposures. They may be discussed as follows:

* t for $P @ .05$.

† For proof of identity, W. H. Beckett in Vol. IX, pp. 10 of "Tropical Agriculture," 1932, refers to Journ. Royal Stat. Soc. XCIV, Part II, "Mathematical theorem involved in the analysis of variance."

TABLE I

ERRORS IN BORDER ROWS (EXPERIMENT 110)

$$\Sigma e = \Sigma [(x - \bar{x}) - (x_1 - \bar{x}) - (x_b - \bar{x})]$$

GROUP I	Plot	Rows	Row yield (x)	General mean (x)	Treatment mean (x ₁)	Block mean (x _b)	e
Rows with no nitrogen adjacent to rows with high nitrogen	55	8	94	104	79	118	+ 1
	57	1	104	104	79	108	+21
	57	8	108	104	79	108	+25*
	26	1	103	104	79	96	+32*
	10	1	85	104	79	86	+24
	10	6	81	104	79	86	+20
	31	6	131	104	79	107	+49*
GROUP II							
Rows with high nitrogen adjacent to rows with no nitrogen	56	1	151	104	128	118	+ 9
	56	8	148	104	128	118	+ 6
	58	1	154	104	128	108	+22
	25	9	176	104	128	96	+56*
	9	6	157	104	128	86	+47*
	32	1	169	104	128	107	+38*
GROUP III							
High N adjacent to high N	25	1	124	104	128	96	+ 4
	9	1	103	104	128	86	- 7
	32	6	133	104	128	107	+ 2
GROUP IV							
No N adjacent to no N	26	9	89	104	79	96	+18
	31	1	83	104	79	107	+ 1
GROUP V							
High N adjacent to unplanted row	58	8	121	104	128	108	-11
GROUP VI							
No N adjacent to unplanted row	55	1	129	104	79	118	+36*

Expected e = $2.01 \times 12.4 = 24.9$ tons. * = significant.

The 7 border rows in Group I, none of which had received any nitrogen fertilizer but were adjacent to cane rows which had been heavily fertilized with nitrogen, all had positive amounts of error which in three cases were greater than would normally be expected by chance; hence this would appear as fairly reliable confirmation of a border effect on cane in these nitrogen-deficient border rows.

Three of the 6 rows in Group II, rows which had been fertilized with nitrogen and which adjoined non-fertilized rows, had errors which were in excess of the expected chance error; hence this fact also supports other evidence of border effect on the high-nitrogen-fertilized rows.

In Groups III and IV there is no statistical proof of border effect and this is to be expected since the border rows are adjacent to rows of cane which had been similarly fertilized.

Data in Groups V and VI are too scanty for discussion but it would certainly appear that row 1 in plot 55 derived some benefit from having the unplanted row adjacent.

Our second study was made on cane cut from Waipio Experiment 114. This test was located on a very uniform piece of land in Field 21. Sixteen 6-row plots were carefully planted with 32-8560 cane on August 4, and thereafter handled to assure as uniform growth as possible. Two nitrogen differentials were used, according to the following plan of fertilization:

Plots	Pounds per acre			Total
	Sept. 24	Nov. 22	March 23	
L	40	40	40	120
H	40	80	80	200

At no time during the growth of this crop were growth differences observed although the general color of the "H" plots may have been a slightly darker green during the first summer; however, this color difference was never clearly apparent in the border rows of adjacent plots.

At harvest* the rows in this test were cut separately and all cane weighed in the field. From these weights, Fig. 2 has been prepared with all cane weights calculated to their equivalent T.C.A. (net area basis). This graph vividly shows the possible nature and extent of the yield variation which may be found in adjacent single rows of 32-8560 plant cane, which in its early growth stages was remarkably uniform and certainly showed no evidence to indicate such extremes of variation as were actually measured when harvested at 22 months. Such facts as these should make quite clear what we have repeatedly emphasized—that yields from single rows of cane are wholly unreliable for comparative purposes because they are the result of so many unknown influences.

Careful study of Fig. 2 will show that the T.C.A. differences between the border row (No. 1 or No. 6) and the adjacent or next inside row (No. 2 or No. 5) in these plots are perhaps no more variable than the differences between some of the inside adjacent rows which are positionally removed from border effect. This makes it difficult to support any great probability that border effect has been a factor in this test. Perhaps the nature of the plan of fertilization was not conducive to border effect for unlike the large differentials in the nitrogen fertilization of Experiment 110 which we have already discussed, the treatment differences in this Experiment 114 were quite small; here it was not a case of "with or without" nitrogen but rather a case of "some and more" and apparently "some" was enough, for we found no real gain for "more" nitrogen (Treatment H) when the test was finally harvested.

A statistical study of these row yields made as previously described for Experiment 110, gives us a high figure of 20.7 tons for the standard deviation of the 64 inside cane rows in this area and indicates that in this 160-ton cane an individual row error of 41.6 tons could be expected for any single row within a similar population of cane stalks. The individual errors of all border rows in this area have been calculated and appear in Table II; they are broken down into 6 groupings to separate the differences in their exposures.

The 10 rows in Group I had each received the low or 120-pound application of nitrogen and were adjacent to rows which had been given the higher level or 200 pounds. It was in this group especially that we had expected that a border effect might be involved which would tend to make the plot yields higher when roots from

* Under careful supervision of Messrs. Smith, Swezey, Yamasaki, and Hind.

the cane in the border rows "stole" nitrogen from the more heavily fertilized row of the adjacent plot. The results can scarcely be said to have verified this expectation for only in Plot 49, row 1, do we note an amount of positive variation ($e = +52$) from the expected (41.6) which might indicate a border effect of this nature. On two other plots, Nos. 47 and 55, an effect of some nature other than chance is suggested which has apparently reduced the yield of their No. 6 or border row.

TABLE II
ERRORS IN BORDER ROWS (EXPERIMENT 114)
 $\Sigma e = \Sigma [(x - x) - (x_t - x) - (x_b - x)]$

GROUP I	Plot	Rows	Row yield(x)	General mean(x)	Treatment mean(x_t)	Block mean(x_b)	e
Rows with low nitrogen adjacent to high nitrogen	42	1	124	160	157	138	-11
	43	6	151	160	157	151	+ 3
	46	1	131	160	157	156	-22
	47	6	131	160	157	179	-45*
	49	1	183	160	157	134	+52*
	49	6	144	160	157	134	+13
	52	1	185	160	157	182	+ 6
	52	6	157	160	157	182	-22
	54	1	146	160	157	150	- 1
	55	6	134	160	157	190	-53*
GROUP II							
Rows with high nitrogen adjacent to low nitrogen	41	6	157	160	163	138	+16
	44	1	157	160	163	151	+ 3
	45	6	129	160	163	156	-30
	48	1	153	160	163	179	-29
	48	6	155	160	163	179	-27
	50	1	128	160	163	134	- 9
	51	6	126	160	163	182	-59*
	53	1	155	160	163	150	+ 2
	53	6	165	160	163	150	+12
GROUP III	56	1	173	160	163	190	-20
Rows with low nitrogen adjacent to crop cane	42	6	209	160	157	138	+74*
	43	1	130	160	157	151	-18
	54	6	182	160	157	150	+35
GROUP IV							
Rows with high nitrogen adjacent to crop cane	41	1	142	160	163	138	+ 1
	44	6	205	160	163	151	+51*
	50	6	141	160	163	134	+ 4
GROUP V							
Rows with low nitrogen adjacent to ditch	47	1	163	160	157	179	-13
	55	1	166	160	157	190	-21
GROUP VI							
Rows with high nitrogen adjacent to ditch	45	1	162	160	163	156	+ 3
	51	1	188	160	163	182	+ 3

Expected $e = 2.01 \times 20.7 = 41.6$ tons. * = significant.

In Group II we have 10 rows fertilized with high nitrogen which adjoin low nitrogen plots. Row 6 in plot 51 has an error which is considerably larger than it should be and this together with the record of its low yield might lead us to imply that it had lost some of its nitrogen to the adjacent row 1 of plot 52, if it were not that we have already seen from Group I that this row was apparently not benefited. Hence it would not be wise to assume any proved border effect for rows in this group.

In Groups III and IV there is no reason for any border effect since the fertilization of the crop cane was identical with that of the border row in the test plots which adjoin it. Hence the reason for the high error in row 6 of both plots 42 and 44 is purely speculative, but indicative of a faulty separation of cane by the individual cutter who harvested both of these rows.

The data in Groups V and VI are too scanty for discussion but are offered at this time merely for record.

The evidence from these 2 tests is somewhat contradictory but we believe that the difference in their 2 plans of fertilization supplies the reason. Thus when large cane yield differences are expected, such as would be the case when a real deficiency in some growth factor exists, there is a good likelihood that border effect will influence yield comparisons, but where expected yield differences are apt to be small, such as would be the case in most of our Grade A "Amounts" tests, this border influence may not be a very large one even if it exists at all. However, the best procedure is to admit the possibility of its existence and to use those features of an intelligent experimental technique that will tend to reduce border effect to a minimum.

Mosquitoes and Some Other Noxious Flies That Occur In New Caledonia

By FRANCIS X. WILLIAMS

Eleven species of mosquitoes are now recorded from New Caledonia. These are discussed in the present paper as well as the occurrence of certain other noxious flies found there.

In May 1940 the Experiment Station of the Hawaiian Sugar Planters' Association sent the writer to New Caledonia to investigate the insects that affect agriculture and the health of man as well as to add data on the general geographical distribution of insects in the Pacific. The information thus acquired during a four-month stay on this island has a direct bearing on the Hawaiian problem of quarantine against foreign insects. With air and sea traffic on the increase, it will become more and more difficult to prevent the accidental introduction of insect pests to our shores. Some of these new pests, should they reach Hawaii alive, would find very suitable breeding places. This is particularly true of mosquitoes, and while malarial mosquitoes (*Anopheles*) seem to be absent in New Caledonia itself—although present in the New Hebrides some 200 miles or more to the north and eastward—other and very annoying species occur there but not in Hawaii. Two species of salt marsh or lagoon mosquitoes, viz. *Aedes vigilax* and *Culex sitiens* are at least at times very common about Noumea, the capital city and chief seaport of New Caledonia. Were these two mosquitoes introduced here they might readily become established in such a large area as Pearl Harbor and in numerous other places along our shores.

New Caledonia—area 6,296 square miles—is an elongate and rugged island that lies just within the Tropic of Capricorn in the southwest Pacific. Many localities on it were visited. I went as far north as Nepoui on the west coast and Hienghene on the east, and made a brief stop at the Isle of Pines, off the southern extremity. More time was spent about Noumea, well to the south, than anywhere else. The collections are far from complete; they were made during the cooler part of the year, some of the visits were short, and the more tropical northern extremity of the island was not seen.

In 1922 Edwards (10) listed and discussed 8 species of mosquitoes from New Caledonia as follows:

Mucidus kermorganti (Laveran)
Aedes (Stegomyia) argenteus Poiret*
Aedes (Ochlerotatus) vigilax (Skuse)
Aedes (Finlaya) notoscriptus (Skuse)
Taeniorhynchus (Coquillettidia) brevicellulus Theo.
Culex sitiens Wied.
Culex fatigans Wied.*

* Also found in Hawaii. *Culex fatigans* is the same as *Culex quinquefasciatus*, and *Aedes argenteus* is the same as *Aedes aegypti*.

Rachionotomyia caledonica, sp. n.

Since that time three others have been definitely recognized or added, making a total of 11 species, viz:

Mucidus alternans (Westw.)

Aedes (*Aedomorphus*) *vexans* (Meig.)

Culex (*Neoculex*) *pseudomelanoconia* Theo.

Nine species, including a new record, were taken on the present survey. Most of these mosquitoes are found elsewhere and some are widely distributed. *Mucidus kermorganti* Laveran has thus far been recorded only from New Caledonia, although it may be but a variety of another species of wider distribution (Edwards 11), while *Tripteroides caledonica* (Edwards) occurs also in the New Hebrides. I, myself, took no *Anopheles*, or malarial mosquitoes, in New Caledonia nor heard of any case of their presence there.

Much of the literature referring to mosquitoes chiefly of the Australasian and Oriental regions has not been available, but those works that were consulted are listed at the end of this paper.

There are many types of breeding places for mosquitoes in New Caledonia. Particularly on the west coast there are mangrove swamps that have occasional stagnant pockets devoid of fish. In the yards and gardens one may find barrels and tanks harboring mosquito larvae. More or less temporary, grass-margined pools occur among the fore hills and roadsides and both clear and sluggish streams are to be found as well as fresh-water swamps. There are also some water-holding plants such as *Nepenthes* wherein the larvae may breed.

Some of the mosquitoes in the collection are reared specimens and it is believed that all of these are correctly associated in regard to their immature and adult stages. All the specimens were determined by the writer.

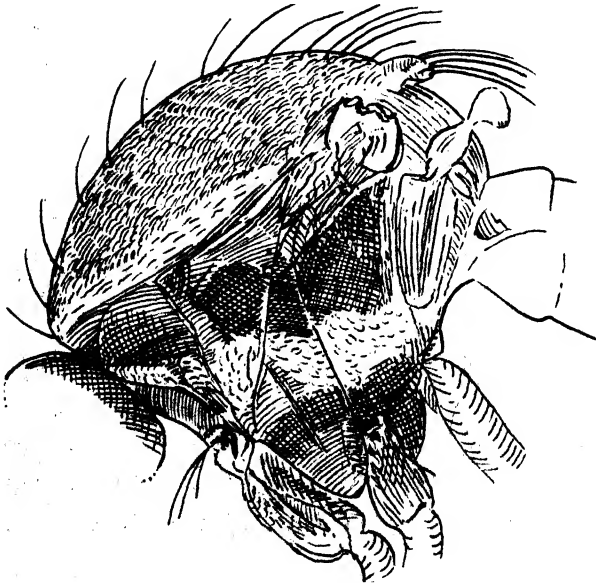


Fig. 1. *Tripteroides caledonica*, female. Side view of thorax. Most of the bristles are omitted.

Tripteroides (Mimeteomyia) caledonica (Edwards)

Rachionotomyia caledonica Edwards, F. W., (10, pp. 100–101), 1922. New Caledonia: Houailou (Montague), June and July 1914, bred from pitcher of *Nepenthes*.

This is a rather slender, neat-looking mosquito with a very long dusky proboscis. It is not heavily scaled and the brownish thorax has two wide stripes of narrow white scales on the side (Fig. 1), the upper stripe being joined in front; the abdomen is nearly black with narrow white bands and the legs except at the base are uniformly dusky. This insect stands or rests quite steeply, head downwards in much the same way as does *Anopheles*.

A single female of this mosquito was taken at Hienghene, well up the east coast, on October 5, 1940. This locality is near Houailou where this species was first taken by Montague in 1914. A fair series was bred in late October and early November 1940, from the pitchers of a species of terrestrial *Nepenthes* plant growing as a



Fig. 2. A species of pitcher plant (*Nepenthes*) on a rocky hillside of southern New Caledonia. Near the center of the picture are three "pitchers", one clearly showing the lid, while somewhat to the left is a pitcher placed in a large vial. The mosquito, *Tripteroides caledonica*, breeds in the liquid in the base of the pitchers. Photo by Louisa Clark Williams.

small colony on a sheltered slope of a ridge leading to a lighthouse at Prony Bay, at the southern extremity of the island. These low plants (Fig. 2) with a portion of their leaves modified pitcher-like, gracefully cylindrical and from $2\frac{1}{2}$ to $5\frac{1}{2}$ inches tall, also bear normal leaves, and flowers on separate stalks. The pitchers contained a rather syrupy liquid in their basal part, including the stem for some distance. *Tripteroides caledonica* larvae and pupae of pale color were active in this liquid. In addition, a number of other insects and a small skink lizard had been trapped in the smooth-walled containers, and had perished and in many cases were well on the way to disintegration in the plant's fluid. Conspicuous among the trapped insects were the ordinary hive bee (*Apis mellifera* L.), some of the smaller native bees, two small spider wasps (Psammocharidae), an ichneumonid wasp, ants, muscoid flies, and a green lacewing (Chrysopidae). In a small forest down the slope was a climbing *Nepenthes* with larger pitchers and likewise containing mosquito young.

Buxton and Hopkins (4) refer to this species (*Rachionotomyia caledonica*) as common in the northern part of the New Hebrides. Details of the anatomy of the larvae (Fig. 3) taken by me from *Nepenthes* plants at Prony Bay do not altogether agree with their figure of the terminal segment (Text Fig. 18, A) but this species

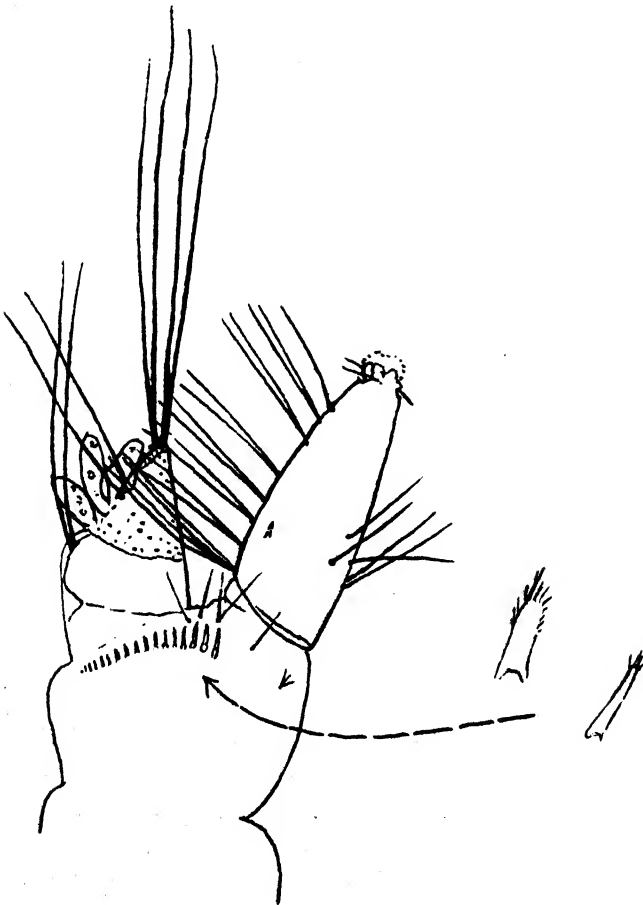


Fig. 3. *Tripteroides caledonica*, larva, tail end.

is admittedly subject to some variation in the larval stage. In my specimens the pecten or the breathing tube is very incomplete, the spines perhaps having been rubbed off. In the New Hebrides the authors above mentioned (4) found this species—identified by F. W. Edwards—breeding in tree holes containing water, i.e. p. 76 “. . . in large numbers in a narrow, deep cavity between the main branches of a *Poinciana* tree”. Elsewhere, species of *Tripteroides* (*Rachionotomyia*) have been found breeding in *Nepenthes*, in tree holes, in an “Old coconut husk with the top cut off for drinking” (Paine 34), in bamboo stubs, in an old kerosene tin, etc. Here they are often associated with other mosquitoes.

Lloyd in his book *The Carnivorous Plants* (28) says, p. 51, “The species of *Nepenthes* are found scattered throughout the tropics of the Old World with the center of distribution in the region of Borneo, being found as far East as N. Australia and New Guinea, and to the West in Ceylon and Madagascar, its extreme outpost (Danser)”. A number of species of insects are associated with these interesting plants; to further quote Lloyd, p. 78, “The nepenthebionts include the remarkable number of 26 species; of the *Phoridae* 6, *Chironomidae* 1, and of the *Culicidae* 19.” “They feed on the animal detritus found there.” At Singapore, Edwards (14, p. 337) found 16 species of mosquitoes, representing 3 genera breeding in pitcher plants.

Lloyd gives a good bibliography on *Nepenthes*.

Mansonia (*Coquillettidia*) *brevicellulus* (Theobald)

Tacniorhynchus brevicellulus Theobald, F. V., Mon. Cul. II, p. 212, 1901.

(Recorded by Theobald as *Chrysoconops acer* Walk.)

This is a moderately large pale brownish, naked-looking mosquito with the abdomen showing a purple gloss. Specimens were collected near St. Louis, New Caledonia in July. Most of them were flushed from a grassy swamp. This species occurs in Papua, New Caledonia, Fiji, and the Oriental Region (Taylor 37). The early stages were not found but it is known that the larvae of *Tacniorhynchus* insert their sharply pointed siphon into the tissues of aquatic plants and thereby obtain oxygen without having to come to the surface.

Mucidus alternans (Westwood)

Culex alternans Westwood, D. O., 1835. Ann. Soc. Ent. France, IV, p. 681, (Nova Hollandia).

Mucidus alternans (Westwood). “New Caledonia, Noumea (J. J. Walker, 1 ♀ in British Museum)”.

Edwards (11) in writing of this species says (p. 367), “A large species which could not be confused with any other in the Australian fauna, owing to the shag-gily-scaled legs with white rings on the tibiae as well as the tarsi. Larvae in shallow swamps.”

Cooling (5) who observed this mosquito in Queensland says, p. 18, “Almost invariably are the larvae of this species to be found in salt marshes.” “The larvae of *M. alternans* are inordinate in their ‘cannibalistic’ desires, for they will greedily devour the larvae of their own species if other larval forms are not available.” This desirable habit goes no farther however, for as an adult this day-flying species is

"... famous for its biting powers ..." (Froggatt 19, p. 290). This conspicuous species was not taken by me.

The larva is well figured by Cooling (5) and by Woodhill and Pasfield (41). The siphonic index (the ratio of the length of the siphon—minus the terminal valves and the acus—to its width at the base) is 3.1–3.3 (Cooling 5), and the anal gills are small and narrow.

Mucidus kermorganti (Laveran)

Culex kermorganti Laveran, C. R., LIII, p. 568, 1901

Edwards (11, p. 367), "New Caledonia: Noumea (Laveran); Calama (Delacour)."

Edwards thinks this may be a form of the preceding. It has not been taken outside of New Caledonia. Not taken by the writer.

Aedes (Stegomyia) aegypti (Linnaeus)

Linnaeus 1762. Hasselquists' Reise nach Palestina, p. 470 (*Culex*). (= *Culex argenteus* Poiret, 1787; *Culex fasciatus* Fab. 1805; *Culex calopus* Meig. 1818; etc.)

Edwards (10) lists this species in his Culicidae of New Caledonia, (*A. argenteus*). The present writer took five males and twelve females of this widely distributed yellow fever and dengue-spreading mosquito, all from Noumea, the majority being taken indoors, July, August, and September 1940.

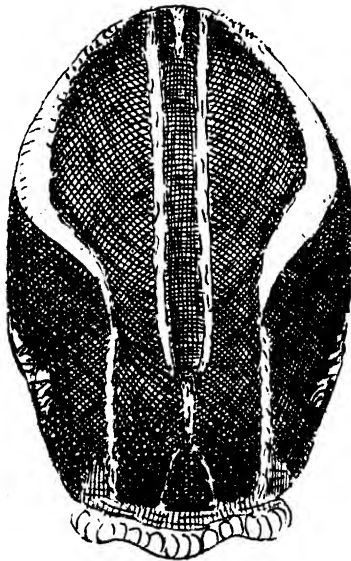


Fig. 4. *Aedes aegypti*, thorax, dorsal view. Noumea, New Caledonia.

This is the species with the silvery lyre-form markings on the top of the thorax (Fig. 4). In and about habitations it commonly breeds in rain-water tanks, saucers of flower pots and other artificial containers; it also breeds in water-holding plants

and in tree cavities. The eggs of *aegypti*—as well as others of the same genus—are known to be drought resistant over a long period. The larva has the comb scales with strong lateral barbs. Buxton and Hopkins (4, p. 114) consider *Aedes aegypti* as well as *Culex quinquefasciatus* (= *fatigans*) to be recent immigrants in Melanesia and Polynesia.

Aedes (Finlaya) notoscriptus (Skuse)

Culex notoscriptus Skuse. Proc. Linn. Soc. N.S.W., (2) III, p. 1738, 1889.
Described from New South Wales, Australia.

Edwards (10, p. 100) records, "A single female was collected by Mr. Montague 15 miles inland on the Houailou River."

The writer collected two females at light at Noumea, September 27, 1940.

The back of the thorax is marked lyre-like somewhat as in *A. aegypti* but lines are much finer and there is only a single median line (Fig. 5). The scales comprising the larval comb are apically rounded and finely fringed (Woodhill and Pasfield 41, p. 204), (Cooling 5, p. 23). Its breeding habits are much as in the preceding species.

A. notoscriptus is well-distributed over the Australian region.

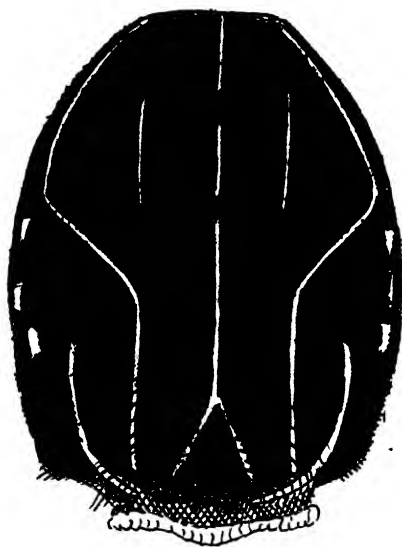


Fig. 5. *Aedes notoscriptus*, thorax, dorsal view. Noumea, New Caledonia.

Aedes (Aedomorphus) vexans (Meigen)

Culex vexans Meigen, Syst. Besch. VI, p. 241, 1830.

Ochlerotatus vexans Edwards, Bull. Ent. Res., VII, pp. 218–219, 1917.

This is a non-domestic nocturnal species having a wide range in tropical and temperate countries. A single pair was taken at Noumea in 1940. Larvae were found in a pool in the hills behind Noumea. Of this species in Fiji, R. W. Paine (34) says, p. 22, "It breeds in puddles on the ground which accumulate after heavy

rain and which, in the writer's experience, are always edged by grasses or other short forms of vegetation . . ."

This mosquito considerably resembles *Aedes (Ochlerotatus) vigilax* Skuse, a common and annoying salt marsh mosquito also occurring in New Caledonia. The male *vexans* is readily separable from *vigilax* by hypopygial characters (Fig. 6). I have seen no male *vigilax*. The single female *vexans* taken has the proboscis prac-



Fig. 6. *Aedes vexans*, aedeagus. Noumea, New Caledonia.

tically unbanded though largely pale-scaled beneath (Fig. 7, B), the vertex of the head has much fine pale hair and fewer dark upright forked scales while there are practically no pale scales on the wings, as are present, to the contrary, on the wings of *vigilax*. The pale abdominal bands hardly show the bilobed condition, as described for typical *vexans* females. The larva of *vexans* has been figured by a number of authors and is readily separable from other species known from New Caledonia, etc., by the two distal scales of the pecten being rather larger and isolated (Fig. 8). The anal gills are long and pointed.

Aedes (Ochlerotatus) vigilax (Skuse)

Culex vigilax Skuse, Proc. Linn. Soc. N.S.W., (2) III, p. 1731, 1889.

Edwards, F. W., Bull. Ent. Res., XIV, p. 375, 1924.

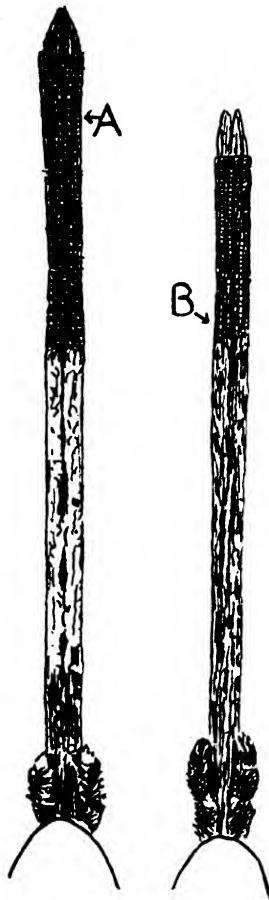


Fig. 7. A—*Aedes vigilax*, female, proboscis, from above. La Foa, New Caledonia. B—*Aedes vexans*, female, proboscis, from above. Noumea, New Caledonia.

At the time of our visit this was the most numerous and annoying of all mosquitoes, biting so persistently during the daytime as to make the collecting of insects—other than its own species—along the seashore around Noumea, a very interrupted procedure.

R. Hanlyn-Harris (20) says, on page 229, "*Aedes vigilax* is the chief long-distance mosquito in Australia and it outnumbers any other pest mosquito in the coastal areas." I found it breeding in a stagnant part of a mangrove swamp at Noumea where it was associated with *Culex sitiens* and a species of *chironomid* fly. Buxton and Hopkins (4) found this species very common and troublesome both day and evening, June 4 and 5, 1925 at Tontouta, on the west side of New Caledonia. It is chiefly an outdoor species.

The larva has very short rounded anal gills (Fig. 9, A)—a character of salt marsh mosquito larvae (Marshall 30, p. 52). The siphon is short, the index as illustrated being approximately 2; this is somewhat greater than that given by Wood-

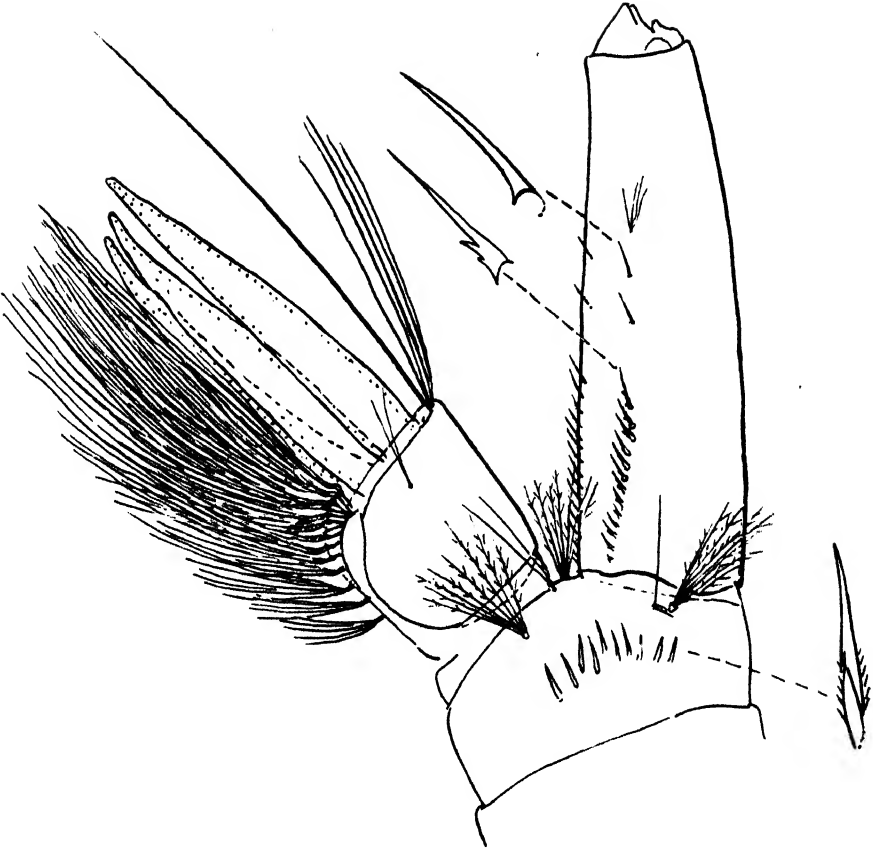


Fig. 8. *Aedes vexans*, larva, tail end. Noumea, New Caledonia.

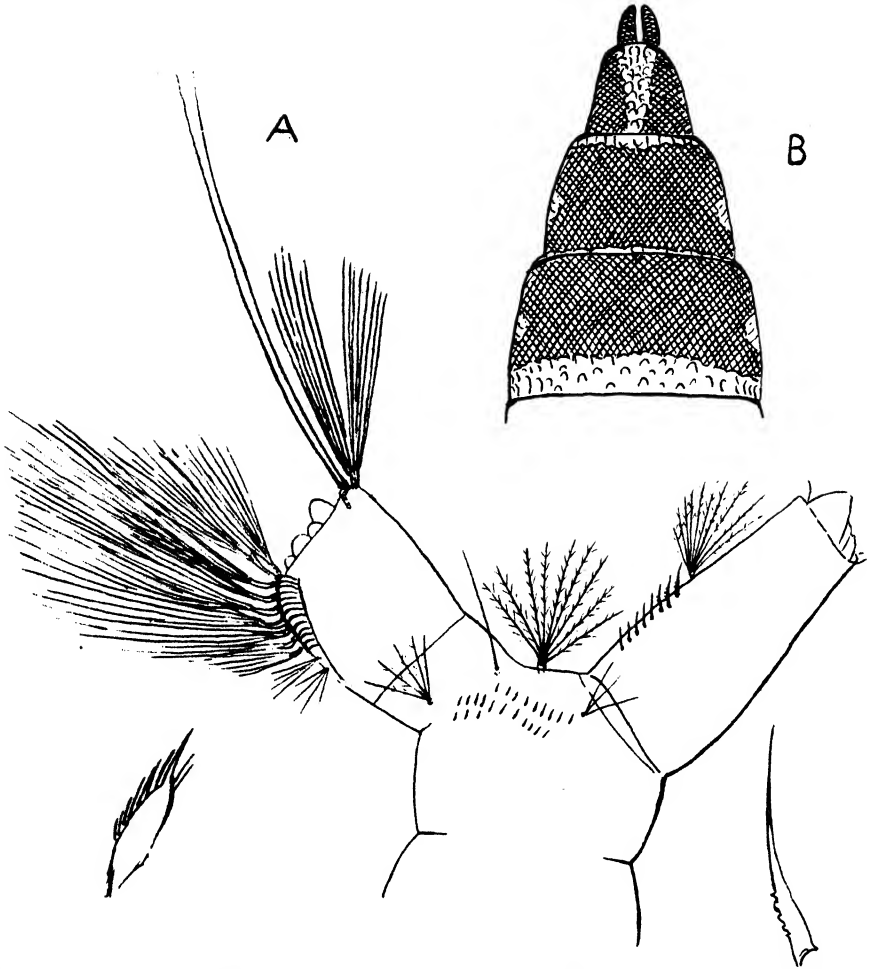


Fig. 9. A—*Aedes vigilax*, larva, tail end. B—female, end of abdomen, from above. Noumea, New Caledonia.

hill and Pasfield (41) and by Cooling (5), and much greater than that given and figured by Brug (2) referring to Dutch-East-Indian mosquitoes—the siphon being "... as long as broad ..."

Hamlyn-Harris (20, pl. vi) gives an excellent figure of the adult, which shows the well-banded legs and the broadly though not very sharply banded proboscis. There is a sprinkling of pale scales on the wings. In Taylor's check list (37) the distribution of *vigilax* is given as "Coasts of Australia, Papua, New Caledonia, Philippine Islands." It has recently been reported by Lever from Fiji (34, pp. 24 and 25, in Paine's "Mosquitoes of Fiji"). The wing denuded of scales and hair is shown in Fig. 10, and the proboscis in Fig. 7, A.

Culex (Culex) sitiens Wied.

Culex sitiens Wiedemann, Aussereurop. Zweifl. Ins. (p. 543), 1828.

Culex sitiens, Edwards, F. W., Bull. Ent. Res., XIV, p. 394, 1924.

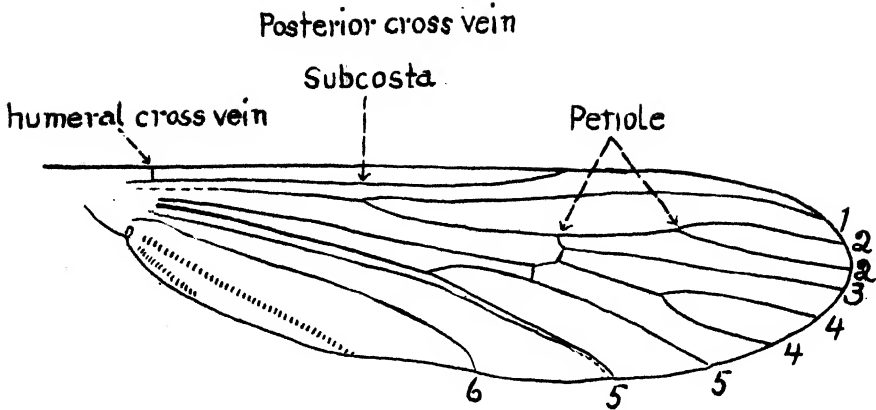


Fig. 10. *Aedes vigilax*, female wing, to show venation.

Buxton and Hopkins, Res. in Polyn. and Melan., p. 79, 1927.

Culex jepsoni Theobald, Ent. XLIII, p. 158, 1910 (Fiji).

MacGregor, M. E., Mosquito Surveys, pp. 167-171, 1927 (quotes Wiedemann's description, etc.)

Somewhat resembling *A. vigilax* but differing from that species in having a strong rather narrow white band on the proboscis (Fig. 11, A) and the legs less conspicuously white-banded, etc. It is a night-biting, often domestic, mosquito that was found associated with *A. vigilax* in the brackish water of a mangrove swamp at

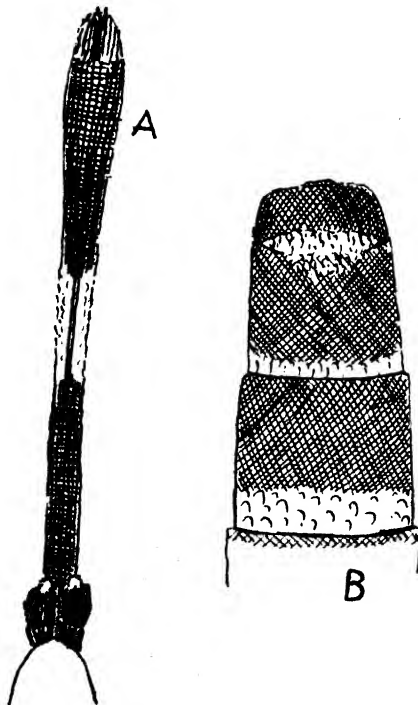


Fig. 11. *Culex sitiens*, female. A—proboscis, from above. B—end of abdomen, from above. Noumea, New Caledonia.

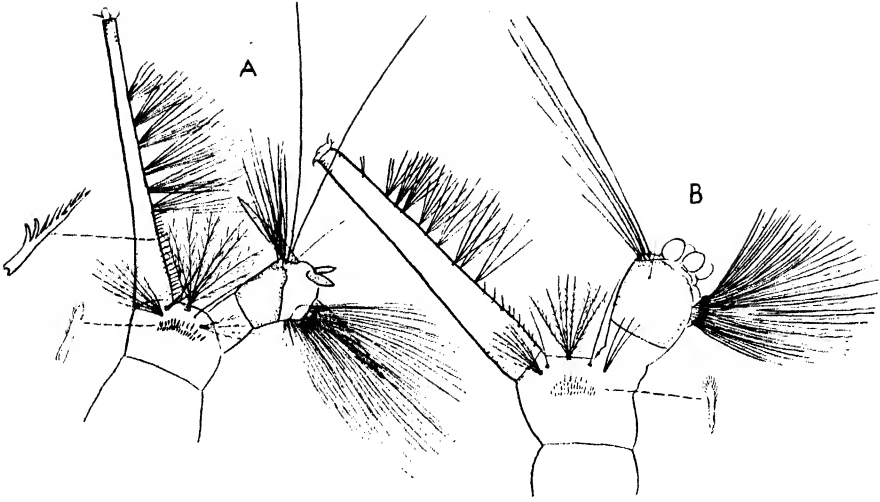


Fig. 12. A—*Culex pseudomelanoconia*, larva, tail end. Ba-Houailou, New Caledonia. B—*Culex sitiens*, larva, tail end. Noumea, New Caledonia.



Fig. 13. *Culex sitiens*, female, aedeagus. Noumea, New Caledonia.

Noumea. Adults in the Experiment Station, H.S.P.A. collection are represented by one male and eleven females. The larva has a siphonal index of about 7.1 (Fig. 12, B) and short rounded anal gills. It is figured by Cooling (5) with the siphonal index of 4.5–5.5. Others give the siphonal index as still shorter so that one wonders if the determination of this species is correct. The aedeagus is shown in Fig. 13.

A widely distributed species.

Culex (Culex) quinquefasciatus Say

Culex quinquefasciatus Say, T., Journ. Acad. Nat. Sci. Phila., III, p. 10, 1823.

Culex fatigans Wiedemann, Auss. Zweifl. Ins., 1, p. 10, 1828.

Culex quinquefasciatus. Dyar, H. G., The mosquitoes of the Americas, Carnegie Insti. of Washington, 1928 (pp. 380–383).

Noumea and St. Louis; larvae, Noumea; in tank in garden. Oua Tom; stagnant pool.

The siphonal index (Fig. 14) of this widely distributed night species varies from 3.4 to 6.5 (Woodhill and Pasfield 41, p. 212).

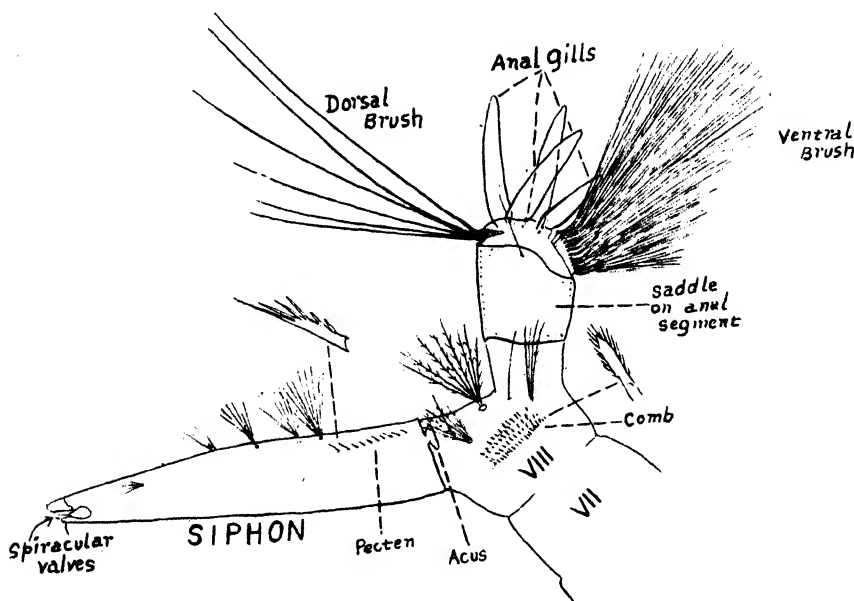


Fig. 14. *Culex quinquefasciatus*, larva, tail end.

Culex (Neoculex) pseudomelanoconia Theobald

Culex pseudomelanoconia Theobald, F. V., Mon. Cul. IV, p. 416, 1907.

Five females bred from larvae in small rock pools of Ba-Houailou stream, just below the beautiful falls and pool. The larva has a very slender anal siphon and rather long dagger-like anal gills (Fig. 12, A).

A small dark, nearly concolorous species described from South Queensland, Australia. Apparently the first record for New Caledonia.

Although sooner or later the malarial mosquito *Anopheles (Myzomyia) punctulatus* Dönitz may be found in New Caledonia, I am unable to discover, at the time of this writing, any evidence of its presence there.

The presence or absence of *Anopheles* in New Caledonia has been the subject of some discussion, and the reader is referred to Taylor (37), Buxton (2a, p. 84), Neveu-Lemaire (33, pp. 1197 and 1198), Zimmerman (42, pp. 296 and 297), Mumford (32) and Lever (25 and 27).

SIMULIDAE (Black Flies)

These are small thickset and usually dark-colored flies with broad wings, the veins of which are strongly developed only towards the anterior or costal border. They breed in streams, requiring rapidly running water for their early stages. Some are great pests often occurring in enormous numbers, and their bites are very irritating. They range well out in the Pacific, occurring in Fiji, the Marquesas and Society Islands, though not occurring in Samoa and the Hawaiian Islands.

The writer found a few specimens of an unidentified species, less than two millimeters long, and apparently a new record for New Caledonia. A note on this fly reads as follows: "Yahoue Valley, August 29, 1940: While seated in the forest sorting out a catch of insects, some *Simulium* flies alighted on my leather jacket and made as if to bite it, mistaking it perhaps for the skin of some large quadruped." One or two other specimens were taken on a mountain height known as Dzumao, farther inland from Noumea. These flies were not troublesome.

Species of Simuliidae, however, are known to be intermediate hosts for certain diseases of man and other animals (Herms 22, pp. 140-142).

TABANIDAE (Horseflies)

Several species of these large flies are found in New Caledonia. They seemed to be coming into season in late October and in November. *Tabanus rubricallosus* Ricardo (Fig. 15), ½-inch long and largely grayish was fairly common about the beaches of the Isle of Pines, at the time of our visit there in late October 1940.

Horseflies are often if not usually vicious biters, attacking man, horses, cattle and other domestic animals. Certain species are sometimes involved in the distribution of anthrax among cattle and sheep and of surra among horses. (See Essig, 21, p. 767, and Edwards, Oldroyd and Smart 16, pp. 75-76.) On the Isle of Pines, New Caledonia, an outbreak of malignant pustules on cattle was suspected of having been transmitted by *Pangonia neocaledonica* Megnin (Tabanidae), and by the ordinary stable fly *Stomoxys calcitrans* (L.). (See Megnin and Germain, 31.)

The larvae of Tabanidae are mainly aquatic, subaquatic, or living in damp soil; they are often found in mud along the edges of ponds and marshes, not excepting salt marshes, some species occurring among seaweed and in the moist sea beach sand or mud.

It is easy to see that certain tabanid flies once introduced, would find suitable breeding places in the lowlands of the Hawaiian Islands.

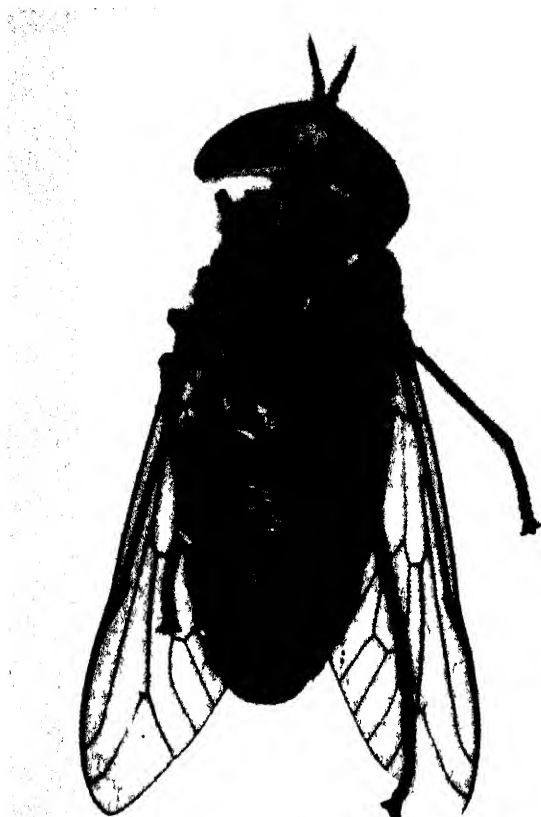


Fig. 15. A horsefly (*Tabanus rubricallosus*) from the Isle of Pines. Length of body 13 mm. Photo by W. Twigg-Smith.

MUSCIDAE
(Houseflies, Etc.)

Musca vicina Macq.

Noumea and Isle of Pines. Not particularly abundant.

The vertex is narrower than in *M. domestica* (see Patton, 1931, II, pp. 595-596).

Stomoxys calcitrans (L.)

The familiar stable fly; Noumea.

I saw no horn flies (*Lyperosia*), a serious pest of horses and cattle in many countries.

HIPPOBOSCIDAE
(Louse Flies, Flat Flies)

Hippobosca equina L.

This flat leathery fly is a pest on horses and cattle, usually preferring the former. It may congregate in large numbers on its host, particularly where the skin

is thinnest and less hairy, as between the hind legs near the tail. It clings very firmly and is dislodged with difficulty.

This fly is viviparous, the egg hatching within the body of the female and developing there until full-grown when it is extruded to pupate immediately. It is common in many parts of the Old World. At present *H. equina* is known in Oceania, from New Caledonia, Loyalty Islands, Fiji, and New Hebrides only (Bequaert 1, p. 258). The writer secured this fly on a horse at Noumea and on a colt on the Isle of Pines.

For further literature relating to this fly see Buxton and Hopkins (4, pp. 56-57), and Edwards, Oldroyd and Smart (16, pp. 122-139).

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The Synthesis of Sucrose in the Sugar Cane Plant—III

The effects of specific inhibitors upon the interconversion of glucose and fructose and the formation of sucrose in detached blades of the sugar cane plant

By CONSTANCE E. HARTT

1. *The use of specific inhibitors:*

The studies presented herein were undertaken for the purpose of learning something of the system whereby sucrose is synthesized in the plant. For this purpose many chemicals have been used, which have been found by other workers to be more or less specific inhibitors of physiological processes. Many investigators have used chemicals considered to be specific inhibitors of physiological processes as tools for elucidating the mechanism of reactions in plants and animals. One of the objections to the method is that the inhibitor may after all not be specific. The justification of applying the results obtained in studies with one organism to investigations with another organism may also be questioned. In the study reported herein, no attempt has been made to prove the specificity of a given poison for a given reaction or type of reactions. The evidence has been taken entirely from the literature and without exception has come from organisms other than sugar cane. This should be understood at the start, as our aim is not to present definite proof, but to build up a picture of a possible mechanism of the formation of sucrose.

If a poison which is known to inhibit a certain enzyme reaction has no effect upon synthesis, then we may conclude that that particular enzyme reaction does not form part of the mechanism of synthesis. If a poison which is known to inhibit a certain enzyme reaction increases synthesis, then it is possible that that particular reaction constitutes a competitor for glucose, *i.e.*, it uses up the glucose which might otherwise have formed sucrose. But if a poison which is known to inhibit a certain enzyme reaction decreases synthesis, then it is possible that the enzyme reaction is in some way a necessary part of the mechanism of synthesis. Of course another possibility remains, that in addition to its known effect the poison in question may also exert other as yet unknown effects.

The aim is therefore to build up a picture of the effects of poisons, in an attempt to formulate a theory of the mechanism of synthesis of sucrose. Such a theory would then have to be tested by other means.

Since studies reported in the first two parts of this paper indicated that aeration and phosphate are essential for interconversion and synthesis, the poisons used in this investigation have been chiefly chemicals known to inhibit steps in the respiratory processes and in phosphorylation. The following is a list of inhibitors discussed in this report: cyanide, pyrophosphate, azide, 8-hydroxyquinoline, iodoacetic acid, arsenite, selenite, fluoride, malonate, acenaphthene, chloroform, dinitrophenol,

ethyl alcohol, histidine, phloridzin, quinine, urethane, brilliant alizarine blue, rosinduline GG, iodine, silver nitrate, copper sulphate, sodium diethyldithiocarbamate, potassium ferricyanide, thymol, sodium pentachlorophenate, and sodium chlorate.

All of the experiments recorded herein were conducted with blades detached from the plant.

Because inhibiting the formation of fructose diphosphate inhibits the formation of sucrose, whereas inhibiting the breakdown of fructose diphosphate increases synthesis, we are led to conclude that fructose diphosphate is a stepping stone in the formation of sucrose by the sugar cane plant.

2. Cyanide:

Cyanide is perhaps the best known of the respiratory inhibitors. The effects of cyanide have been reviewed by Commoner (9),* Elvehjem (23), Oppenheimer (67), and others. Cyanide forms a complex with ferric iron preventing its reduction to ferrous iron. Since the interchange between ferric and ferrous iron is the essential feature of the action of many of the respiratory catalysts, a large part of the respiration of both plants and animals is sensitive to cyanide. The following enzymes are partially or completely inhibited by cyanide: Warburg's Atmungsferment (= cytochrome oxidase = indophenol oxidase = pheohemin), peroxidases, catalase, uricase, ascorbic acid oxidase, tyrosinase, dopa oxidase, orthophenol oxidase, xanthine dehydrogenase, and probably others. Some types of dihydroxymaleic acid oxidase are inhibited by cyanide, according to Boswell and Whiting (6). That part of respiration involving the yellow enzyme and triphosphopyridine nucleotide is cyanide-stable. Respiration is inhibited by 10^{-4} M CN, and fermentation by 10^{-2} M, according to Oppenheimer. Dixon and Elliott (19) stated that M/1000 CN gave maximum inhibition of respiration of animal tissues. Leloir and Dixon (50) used M/500 CN in their study of dehydrogenases. Commoner found that the percentage of inhibition of respiration of baker's yeast by a given concentration of cyanide is greater in the presence of sugar than in its absence. Leloir and Dixon found that cyanide significantly increased the activity of hexose diphosphate dehydrogenase, an effect which they attributed to the presence of traces of heavy metals. Hexokinase, the enzyme which catalyzes the transfer of phosphate from adenosine triphosphate to glucose, is unaffected by cyanide, according to Case (8). Leonard (51) found that corn blades given 0.001–1.0% KCN or NaCN showed equal synthesis of sucrose from glucose.

Several tests have been conducted in which blades of sugar cane were supplied with cyanide along with glucose, fructose, or both. The results of one experiment, in which 0.049 gms. NaCN per liter were used, are reported in Table I. The gains in sugars and the synthetic efficiencies are shown in Table II. It is evident that

* Numbers in parentheses refer to literature citations at the end of the fourth part of this paper.

TABLE I

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE, FRUCTOSE, OR BOTH, WITH AND WITHOUT 0.049 GMS. NaCN PER LITER, FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	69.64±0.057	0.538±0.008	2.459±0.018	3.126±0.010
Water	70.00±0.081	0.302±0.017	2.247±0.033	2.668±0.018
NaCN	69.20±0.076	0.475±0.032	1.746±0.009	2.313±0.022
Glucose	69.05±0.052	0.817±0.019	4.288±0.010	5.332±0.009
Fructose	68.92±0.067	0.924±0.001	4.541±0.022	5.705±0.023
Both	69.55±0.048	1.095±0.024	4.199±0.021	5.516±0.001
Glucose + NaCN	67.28±0.071	0.828	3.987	5.025
Fructose + NaCN	66.51±0.019	0.875±0.001	4.061±0.013	5.149±0.004
Both + NaCN	67.87±0.019	0.917±0.018	3.977±0.002	5.104±0.021

TABLE II

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE, FRUCTOSE, OR BOTH, WITH AND WITHOUT 0.049 GMS. NaCN PER LITER, FOR 24 HOURS, CALCULATED FROM TABLE I

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Water	-0.458	-0.212	0
NaCN	-0.813	-0.713	0
Glucose	2.206	1.829	82.91
Fructose	2.579	2.082	80.72
Both	2.390	1.740	72.80
Glucose + NaCN	1.899	1.528	80.46
Fructose + NaCN	2.023	1.602	78.84
Both + NaCN	1.978	1.518	76.74

cyanide did not inhibit the synthesis of sucrose. When weaker concentrations of cyanide were used (5-25 p.p.m. NaCN) the synthetic efficiency was generally increased a little, but in a few tests there was a slight decrease. For example, in one test the synthetic efficiencies were as follows: with glucose, 75.34; with glucose + 5 p.p.m. NaCN, 77.89; with glucose + 10 p.p.m. NaCN, 73.00; and with glucose + 25 p.p.m. NaCN, 81.33. The percentages of fructose and glucose in the blades supplied with 0.049 gms. NaCN per liter are presented in Table III, which shows no evidence of inhibition of interconversion of glucose and fructose.

TABLE III

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE, FRUCTOSE, OR BOTH, WITH AND WITHOUT 0.049 GMS. NaCN PER LITER, FOR 24 HOURS

Series	Fructose	Glucose
Initial control	0	0.538±0.008
Water	0.149±0.071	0.183±0.039
NaCN	0.407±0.012	0.068±0.020
Glucose	0.606±0.016	0.211±0.036
Fructose	0.527±0.027	0.397±0.017
Both	0.672±0.027	0.423±0.002
Glucose + NaCN	0.510±0.011	0.329
Fructose + NaCN	0.531±0.124	0.344±0.003
Both + NaCN	0.578±0.171	0.339±0.025

It is evident from these results that cyanide inhibited neither the interconversion of glucose and fructose nor the formation of sucrose. Yet the cyanide was strong enough to inhibit the cyanide-sensitive respiratory processes. It would appear that the Warburg-Keilin system of oxidase and the cytochrome complex is not an essential component of the mechanism of synthesis of sucrose.

3. *Pyrophosphate:*

Pyrophosphate inhibits reactions catalyzed by iron or copper. However, it does not inhibit cytochrome oxidase. It has no effect on the respiration of baker's yeast. Leloir and Dixon (50) found that pyrophosphate strongly inhibits succinic dehydrogenase, but does not inhibit any other dehydrogenase studied. Dixon and Elliott (19) found that 0.01 M pyrophosphate inhibited the respiration of liver 30-70 per cent. The action of pyrophosphate has been studied chiefly with animal tissues, and no paper dealing with the effect of pyrophosphate upon the respiration of higher plants has come to the attention of the author. The effects of pyrophosphate have been reviewed by Elvehjem (23) and Oppenheimer (67).

The effect of pyrophosphate upon the formation of sucrose from glucose was studied in blades supplied with 8 grams sodium pyrophosphate per liter, and the results are recorded in Table IV. The gains in sugars and the synthetic efficiencies are presented in Table V. The percentages of fructose and glucose are reported in Table VI. Pyrophosphate did not inhibit either the conversion of glucose to fructose or the formation of sucrose. This finding indicates that the reactions catalyzed by iron or copper do not take part in interconversion or synthesis. Since succinic dehydrogenase is inhibited by pyrophosphate, and synthesis is not, succinic dehydrogenase is not essential for the synthesis of sucrose.

TABLE IV

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT 8 GRAMS $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10 \text{H}_2\text{O}$ PER LITER, FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	68.24 ± 0.029	0.884 ± 0.009	2.585 ± 0.008	3.606 ± 0.019
Glucose	69.32 ± 0.005	1.773 ± 0.007	6.453 ± 0.014	8.566 ± 0.022
Glucose + pyrophosphate ...	69.37 ± 0.100	1.458 ± 0.011	5.866 ± 0.029	7.634 ± 0.042

TABLE V

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT 8 GRAMS $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$ PER LITER, FOR 24 HOURS, CALCULATED FROM TABLE IV

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	4.960	3.868	77.98
Glucose + pyrophosphate	4.028	3.281	81.45

TABLE VI

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT 8 GRAMS $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$ PER LITER, FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.814 ± 0.029		0.070 ± 0.020	
Glucose	1.197 ± 0.019	0.383	0.576 ± 0.026	0.506
Glucose + pyrophosphate	1.027 ± 0.008	0.213	0.431 ± 0.003	0.361

4. Azide:

Sodium azide (Na N_3) slightly accelerates alpha-phosphoglycerol dehydrogenase, according to Elvehjem (23). Sodium azide is a very strong inhibitor of catalase prepared from horse liver. Keilin and Hartree (43) state that azide stabilizes the reduced intermediate compound and thus inhibits the catalase reaction. It also inhibits the respiration of yeast. Oppenheimer (67) states that azide reversibly inhibits catalase and also inhibits peroxidase, indophenol oxidase and phenolase. Keilin and Hartree used 0.001 gram sodium azide for 2 cc. strong catalase solution.

The effect of sodium azide upon sugar transformations in cane blades was studied using the same strength of azide used by Keilin and Hartree. The results are presented in Table VII. The gains in sugars and the synthetic efficiencies are reported in Table VIII. The percentages of fructose and glucose are recorded in Table IX. Sodium azide did not prevent either the conversion of glucose to fruc-

TABLE VII

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT 0.05 GRAMS NaN_3 PER LITER, FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	66.02 \pm 0.024	0.556 \pm 0.029	2.824 \pm 0.000	3.529 \pm 0.004
Water	67.88 \pm 0.019	0.571 \pm 0.007	2.437 \pm 0.015	3.137 \pm 0.023
NaN_3	67.57 \pm 0.052	0.488 \pm 0.015	2.367 \pm 0.021	2.980 \pm 0.038
Glucose	65.65 \pm 0.057	0.749 \pm 0.008	4.268 \pm 0.016	5.242 \pm 0.008
Glucose + NaN_3	65.96 \pm 0.019	0.997 \pm 0.003	4.770 \pm 0.013	6.019 \pm 0.017

TABLE VIII

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT 0.05 GM. NaN_3 PER LITER, FOR 24 HOURS, CALCULATED FROM TABLE VII

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Water	-0.392	-0.387	
NaN_3	-0.549	-0.457	
Glucose	1.713	1.444	84.29
Glucose + NaN_3	2.490	1.946	78.15

TABLE IX

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT 0.05 GM. NaN_3 PER LITER, FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.144 \pm 0.000		0.412 \pm 0.004	
Water	0.301 \pm 0.001	0.157	0.270 \pm 0.005	-0.142
NaN_3	0.304 \pm 0.000	0.160	0.184 \pm 0.015	-0.228
Glucose	0.282 \pm 0.002	0.138	0.467 \pm 0.009	0.055
Glucose + NaN_3	0.393 \pm 0.009	0.249	0.603 \pm 0.006	0.191

tose or the formation of sucrose, although it did decrease the synthetic efficiency a little. Catalase, peroxidase, indophenol oxidase and phenolase probably play no important part in either interconversion or synthesis.

5. 8-hydroxyquinoline:

This is a copper poison and inhibits the action of enzymes containing or depend-

ing on copper. Elvehjem (23) states that polyphenolases, potato oxidase, phenol oxidase, laccase, catechol oxidase, and tyrosinase are all copper proteinates. Ramasarma (72) says that it also inhibits ascorbic acid oxidase.

Blades were supplied with glucose with and without 8-hydroxyquinoline (25 p.p.m.) for 24 hours, and the results are presented in Table X. The gains in sugars and the synthetic efficiencies are reported in Table XI. The percentages of fructose and glucose are recorded in Table XII. These results show that 25 p.p.m. 8-

TABLE X

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT 25 P.P.M. 8-HYDROXYQUINOLINE, FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	70.65±0.019	0.603±0.019	1.944±0.008	2.649±0.010
Glucose	70.74±0.038	1.206±0.018	5.133±0.006	6.610±0.011
Glucose + 8-hydroxyquinoline	70.19±0.000	1.175±0.000	4.911±0.005	6.344±0.005

TABLE XI

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT 25 P.P.M. 8-HYDROXYQUINOLINE, FOR 24 HOURS, CALCULATED FROM TABLE X

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	3.961	3.189	80.50
Glucose + 8-hydroxyquinoline	3.695	2.967	80.29

TABLE XII

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT 25 P.P.M. 8-HYDROXYQUINOLINE, FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.873±0.009		0	
Glucose	0.490±0.006	-0.383	0.716±0.024	0.716
Glucose + 8-hydroxyquinoline	0.768±0.012	-0.105	0.406±0.012	0.406

hydroxyquinoline did not inhibit either the conversion of glucose to fructose or the formation of sucrose, which indicates that the oxidases mentioned in the preceding paragraph are not essential for conversion or synthesis.

The results with cyanide, pyrophosphate, azide, and 8-hydroxyquinoline agree in showing that the oxidases, peroxidases, and catalase are not required, either directly or indirectly, for the conversion of glucose to fructose or for the synthesis of sucrose.

6. Iodoacetate:

The statement is sometimes made that iodoacetate inhibits fermentation but not respiration. This is not strictly true, as both time and concentration must be considered. Turner (80) states that iodoacetate acts more quickly on fermentation than on respiration. He also states that probably any concentration of iodoacetate which inhibits fermentation will in time also decrease respiration. Data regarding the effect of concentration of iodoacetate upon respiration and fermentation are summarized in Table XIII, which shows that strong iodoacetate inhibits both fer-

TABLE XIII

SUMMARY OF DATA REGARDING EFFECT OF CONCENTRATION OF IODOACETATE UPON RESPIRATION AND FERMENTATION

Concentration	General effect	Author
.01 M	inhibits aldehyde, succinic, lactic, glycerophosphate, fumaric, alcohol and triosephosphate dehydrogenases, and aldehyde mutase	Elvehjem (23)
.01 M-.002 M	inhibits both fermentation and respiration	Nilson (80)
.005 M	inhibits hexokinase almost completely	Iri (40)
.002 M	inhibits hexokinase incompletely	Iri (40)
.00108 M	inhibits fermentation but only slightly depresses respiration	Turner (80)
.001 M	inhibits fermentation in carrot and yeast	Turner (81)
.001 M	inhibits fermentation and decreases respiration 50%	Engelhardt (25)
.001 M	inhibits fermentation	Elvehjem (23)
<.001 M	specifically inhibits alcohol dehydrogenase and triosephosphate dehydrogenase	Dixon (18)
.0001 M	only fermentation inhibited	Nilson (80)
c .001 M-.0001 M	inhibits only fermentation and maintains respiration	Barker (3)

mentation and respiration, but that very weak iodoacetate inhibits only fermentation.

To find the effect of iodoacetate upon sugar conversions in cane blades an experiment was conducted using 0.0001 M iodoacetate to inhibit only fermentation and 0.01 M iodoacetate to inhibit both fermentation and respiration. The iodoacetic acid was dissolved in alcohol and neutralized with sodium hydroxide. The results are presented in Table XIV. The gains in sugars and the synthetic efficiencies are recorded in Table XV. The fructose and glucose percentages are reported in Table XVI. The weak iodoacetate did not inhibit either the conversion of glucose to fruc-

TABLE XIV

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT 0.0001 M AND 0.01 M IODOACETATE, FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	68.56±0.029	0.737±0.013	2.720±0.008	3.600±0.005
Glucose	69.87±0.000	1.228±0.037	4.937±0.058	6.426±0.024
Glucose + .0001 M IAA	67.60±0.000	1.055±0.004	4.876±0.025	6.188±0.022
Glucose + .01 M IAA	69.43±0.014	3.097±0.012	2.726±0.021	5.966±0.034

TABLE XV

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT 0.0001 M AND 0.01 M IODOACETATE, FOR 24 HOURS, CALCULATED FROM TABLE XIV

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	2.826	2.217	78.45
Glucose + .0001 M IAA	2.588	2.156	83.30
Glucose + .01 M IAA	2.366	0.006	0

TABLE XVI

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT 0.001 M AND 0.01 M IODOACETATE, FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.663±0.008		0.073±0.021	
Glucose	0.462±0.008	-0.201	0.766±0.029	0.693
Glucose + .0001 M IAA	0.738±0.058	0.075	0.317±0.054	0.244
Glucose + .01 M IAA	0.701±0.005	0.038	2.396±0.007	2.323

tose or the formation of sucrose; in fact there was a slight increase in synthetic efficiency in the presence of weak iodoacetate. Strong iodoacetate, however, completely inhibited the formation of sucrose. The large accumulation of glucose in the presence of strong iodoacetate, accounting almost entirely for the gain in total sugars, showed that iodoacetate prevented the conversion of glucose to fructose.

The effect of strong iodoacetate was then studied using glucose, fructose, and both of the sugars. The results are shown in Table XVII. The gains in sugars and the synthetic efficiencies are recorded in Table XVIII. The percentages of fructose and glucose are presented in Table XIX. The synthetic efficiency of 9.60, shown in Table XVIII, where the blades were supplied with both glucose and fruc-

TABLE XVII

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE OR FRUCTOSE WITH AND WITHOUT .01 M IODOACETATE, FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	70.02±0.067	0.806±0.001	2.430±0.005	3.364±0.007
Glucose	71.55±0.062	1.599±0.021	4.883±0.011	6.739±0.032
Fructose	70.74±0.005	1.547±0.019	4.662±0.037	6.455±0.020
Both	70.21±0.019	1.388±0.013	4.790±0.039	6.431±0.029
Glucose + .01 M IAA	71.00±0.033	3.248±0.002	2.497±0.069	5.877±0.071
Fructose + .01 M IAA	70.41±0.005	2.609±0.010	2.509±0.014	5.250±0.004
Both + .01 M IAA	69.16±0.009	2.893±0.023	2.653±0.056	5.685±0.082

TABLE XVIII

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE OR FRUCTOSE WITH AND WITHOUT .01 M IODOACETATE, FOR 24 HOURS, CALCULATED FROM TABLE XVII

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	3.375	2.453	72.68
Fructose	3.091	2.232	72.20
Both	3.067	2.360	76.94
Glucose + .01 M IAA	2.513	0.067	0
Fructose + .01 M IAA	1.886	0.079	0
Both + .01 M IAA	2.321	0.223	9.60

TABLE XIX

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE OR FRUCTOSE WITH AND WITHOUT .01 M IODOACETATE, FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.294±0.006		0.511±0.005	
Glucose	0.726±0.079	0.432	0.873±0.058	0.362
Fructose	1.035±0.025	0.741	0.512±0.043	0.001
Both	0.885±0.060	0.591	0.503±0.047	-0.008
Glucose + .01 M IAA	0.813±0.000	0.519	2.434±0.002	1.923
Fructose + .01 M IAA	2.076±0.057	1.782	0.533±0.067	0.022
Both + .01 M IAA	1.660±0.049	1.366	1.232±0.025	0.721

tose in the presence of .01 M iodoacetate is not significant, because the gain in sucrose (0.223) was not significant. These results demonstrate the complete inhibitory effect of strong iodoacetate upon synthesis of sucrose, in blades supplied with

glucose, fructose, or both. Glucose accumulated in the blades supplied with glucose, and fructose accumulated in the blades supplied with fructose, indicating that strong iodoacetate inhibits the interconversion of glucose and fructose. However, something more than just interconversion was inhibited by iodoacetate, for the synthesis of sucrose was inhibited even when both glucose and fructose were supplied.

The factor involved in the inhibition of interconversion and synthesis by strong iodoacetate might be one of the dehydrogenases listed in Table XIII, but not succinic dehydrogenase for that is inhibited by pyrophosphate and sodium diethyldithiocarbamate, neither of which inhibits interconversion or synthesis, as shown in this report. The factor involved might be hexokinase, which catalyzes the transfer of phosphate from adenosine triphosphate to glucose.

7. Arsenite:

Arsenite is a potent inhibitor of respiratory processes. Szent-Györgyi (78) stated that alcoholic fermentation in yeast is practically unaffected by 0.057 M arsenite, a concentration which almost completely inhibits oxygen uptake. Therefore he said that arsenite makes possible a separation of respiration and fermentation by inhibiting respiration. Indophenol oxidase of liver was quite insensitive to a high concentration of arsenite (0.01 M), indicating that arsenite has no effect on oxygen activation. Arsenite had little effect on hydrogen activation either, according to Szent-Györgyi. Pillai (69) stated that arsenic activated the breakdown of hexose diphosphate in muscle extract, and Harden (60) found that it accelerated fermentation in yeast extract by quicker splitting of fructose diphosphate. Elvehjem (23) said that succinic acid dehydrogenase is almost completely inhibited by arsenite, and Oppenheimer (67) stated that arsenic inhibits the enzyme which attacks ketoglutaric acid and blocks the citric acid cycle at that level. Dixon (18) reported that the reaction, triose phosphate \rightarrow phosphoglyceric acid is coupled with the synthesis of adenylypyrophosphate from inorganic phosphate and adenylic acid. This coupling is broken by arsenic. Since adenylypyrophosphate (= adenosinetriphosphate = cophosphorylase) is required both for the phosphorylation of glucose and for the formation of fructose diphosphate from fructose monophosphate, this adds two more steps in intermediate carbohydrate metabolism which may be inhibited by arsenic.

Several concentrations of sodium arsenite have been used in tests with cane blades. The effect of arsenite upon the formation of sucrose from glucose is shown in Table XX. The gains in sugars and the synthetic efficiencies are presented in Table XXI. The percentages of fructose and glucose are reported in Table XXII.

TABLE XX

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT SODIUM ARSENITE (5 P.P.M.-100 P.P.M. As) FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	73.42 \pm 0.043	1.135 \pm 0.005	1.849 \pm 0.008	3.082 \pm 0.013
Glucose	73.11 \pm 0.005	2.030 \pm 0.011	5.851 \pm 0.018	8.189 \pm 0.007
Glucose + 5 p.p.m. As	72.23 \pm 0.071	2.466 \pm 0.017	5.642 \pm 0.002	8.406 \pm 0.019
Glucose + 25 p.p.m. As	71.85 \pm 0.143	4.433 \pm 0.032	3.278 \pm 0.022	7.884 \pm 0.009
Glucose + 100 p.p.m. As	71.60 \pm 0.172	6.151 \pm 0.060	1.837 \pm 0.020	8.084 \pm 0.038

TABLE XXI

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT SODIUM ARSENITE (5-100 P.P.M. As) FOR 24 HOURS, CALCULATED FROM TABLE XX

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	5.107	4.002	78.36
Glucose + 5 p.p.m. As	5.324	3.793	71.24
Glucose + 25 p.p.m. As	4.802	1.429	28.56
Glucose + 100 p.p.m. As	5.002	-0.012	0

TABLE XXII

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT SODIUM ARSENITE (5 P.P.M.-100 P.P.M. As) FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.601±0.006		0.534±0.011	
Glucose	0.721±0.025	0.120	1.309±0.014	0.775
Glucose + 5 p.p.m. As	0.866±0.009	0.265	1.600±0.008	1.066
Glucose + 25 p.p.m. As	0.642±0.000	0.041	3.791±0.032	3.257
Glucose + 100 p.p.m. As	0.485±0.023	-0.116	5.665±0.037	5.132

It is evident that arsenite decreased the conversion of glucose to fructose and the formation of sucrose. One hundred p.p.m. As inhibited these processes 100 per cent.

The effect of arsenite upon the formation of sucrose from fructose is shown in Table XXIII. The gains in sugars and the synthetic efficiencies are recorded in Table XXIV. The percentages of fructose and glucose are reported in Table XXV.

TABLE XXIII

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH FRUCTOSE WITH AND WITHOUT SODIUM ARSENITE (5 P.P.M. As-100 P.P.M. As) FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	74.54±0.081	0.735±0.011	1.541±0.013	2.358±0.002
Fructose	73.59±0.162	1.336±0.013	4.640±0.008	6.221±0.021
Fructose + 5 p.p.m. As	73.53±0.105	1.546±0.023	4.422±0.023	6.200±0.000
Fructose + 25 p.p.m. As	74.08±0.033	2.115±0.019	2.932±0.008	5.202±0.027
Fructose + 100 p.p.m. As ...	72.60±0.091	3.741±0.013	1.506±0.003	5.326±0.009

TABLE XXIV

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH FRUCTOSE WITH AND WITHOUT SODIUM ARSENITE (5 P.P.M.-100 P.P.M. As) FOR 24 HOURS, CALCULATED FROM TABLE XXIII

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Fructose	3.863	3.099	80.22
Fructose + 5 p.p.m. As	3.842	2.881	74.99
Fructose + 25 p.p.m. As	2.844	1.391	48.91
Fructose + 100 p.p.m. As	2.968	-0.025	0

TABLE XXV

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH FRUCTOSE WITH AND WITHOUT SODIUM ARSENITE (5 P.P.M.-100 P.P.M. As) FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.481±0.010		0.254±0.000	
Fructose	1.019±0.050	0.538	0.317±0.037	0.063
Fructose + 5 p.p.m. As	1.053±0.030	0.572	0.492±0.007	0.238
Fructose + 25 p.p.m. As	1.919±0.038	1.438	0.195±0.056	-0.059
Fructose + 100 p.p.m. As	3.861±0.023	3.380	0.000±0.000	-0.254

These results show that arsenic decreases or inhibits the formation of sucrose from fructose and the conversion of fructose to glucose.

The effect of arsenite upon the formation of sucrose when both glucose and fructose were supplied to the blades is shown in Table XXVI. The gains in sugars and the synthetic efficiencies are presented in Table XXVII. The fructose and glucose percentages are reported in Table XXVIII. The effect of arsenite upon

TABLE XXVI

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH BOTH GLUCOSE AND FRUCTOSE WITH AND WITHOUT SODIUM ARSENITE (5 P.P.M.-100 P.P.M. As) FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	75.03±0.005	0.980±0.003	1.367±0.017	2.419±0.015
Glucose + fructose	74.19±0.100	1.688±0.009	4.706±0.017	6.642±0.028
Both + 5 p.p.m. As	72.85±0.124	1.714±0.000	4.382±0.006	6.327±0.006
Both + 25 p.p.m. As	72.33±0.043	2.783±0.030	2.493±0.002	5.407±0.028
Both + 100 p.p.m. As	72.97±0.186	4.175±0.005	1.235±0.031	5.475±0.038

TABLE XXVII

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH BOTH GLUCOSE AND FRUCTOSE WITH AND WITHOUT SODIUM ARSENITE (5 P.P.M.-100 P.P.M. As) FOR 24 HOURS

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose + fructose	4.226	3.339	79.01
Both + 5 p.p.m. As	3.908	3.015	77.14
Both + 25 p.p.m. As	2.988	1.126	37.68
Both + 100 p.p.m. As	3.056	-0.132	0

TABLE XXVIII

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH BOTH GLUCOSE AND FRUCTOSE WITH AND WITHOUT SODIUM ARSENITE (5 P.P.M.-100 P.P.M. As) FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.831±0.059		0.199±0.080	
Glucose + fructose	1.139±0.011	0.308	0.549±0.020	0.350
Both + 5 p.p.m. As	1.166±0.017	0.335	0.548±0.017	0.349
Both + 25 p.p.m. As	1.910±0.030	1.079	0.873±0.000	0.674
Both + 100 p.p.m. As	2.399±0.027	1.568	1.776±0.032	1.577

the formation of sucrose when both glucose and fructose were supplied was very similar to the effect when glucose and fructose were supplied separately. As would be expected, both glucose and fructose accumulated in the presence of arsenite.

The conclusion is drawn that arsenite inhibits both the interconversion of glucose and fructose and the formation of sucrose in blades of the sugar cane plant. The inhibitory effect is found with as little arsenite as 5 p.p.m. Inhibition is complete with 100 p.p.m. As.

8. Selenite:

The effect of selenite was reviewed by Elvehjem (23), who said that succinic acid dehydrogenase is almost completely inhibited by selenite; 0.003 M selenite inhibited the oxidation of glucose, mannose, and fructose 80 per cent, of succinate 73 per cent, of acetate 50 per cent, and of lactate and pyruvate less than 10 per cent.

The effect of selenite upon the transformations of sugars was studied with several concentrations of sodium selenite (5–100 p.p.m. Se) and the results are presented in Table XXIX. The gains in sugars and the synthetic efficiencies are reported in Table XXX. The synthetic efficiency of 3.08 obtained with 100 p.p.m. Se was not significant, because the gain in sucrose (0.115) was not significant. The percentages of fructose and glucose are reported in Table XXXI.

TABLE XXIX

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT SODIUM SELENITE (5–100 P.P.M. Se) FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	74.60±0.013	0.785±0.000	1.690±0.006	2.564±0.007
Glucose	73.09±0.043	1.691±0.004	6.137±0.009	8.151±0.005
Glucose + 5 p.p.m. Se	72.62±0.071	1.641±0.006	5.797±0.001	7.743±0.004
Glucose + 25 p.p.m. Se	72.75±0.086	2.328±0.012	3.969±0.002	6.506±0.014
Glucose + 100 p.p.m. Se	73.24±0.005	4.389±0.016	1.805±0.027	6.289±0.045

TABLE XXX

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT SODIUM SELENITE (5–100 P.P.M. Se) FOR 24 HOURS, CALCULATED FROM TABLE XXIX

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	5.587	4.447	79.59
Glucose + 5 p.p.m. Se	5.179	4.107	79.30
Glucose + 25 p.p.m. Se	3.942	2.279	57.81
Glucose + 100 p.p.m. Se	3.725	0.115	3.08

TABLE XXXI

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT SODIUM SELENITE (5 P.P.M.–100 P.P.M. Se) FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.767±0.040		0.049±0.023	
Glucose	1.033±0.069	0.266	0.658±0.077	0.609
Glucose + 5 p.p.m. Se	0.784±0.000	0.017	0.857±0.007	0.808
Glucose + 25 p.p.m. Se	0.832±0.000	0.065	1.496±0.012	1.447
Glucose + 100 p.p.m. Se	0	−0.767	4.389±0.016	4.340

The effect of sodium selenite (100 p.p.m. Se) upon the formation of sucrose from glucose or fructose or both is shown in Table XXXII. The gains in sugars and the synthetic efficiencies are reported in Table XXXIII. The gains in sucrose in the blades supplied with fructose or both glucose and fructose were practically insignificant, which of course made the synthetic efficiencies calculated for those series also of little significance. The percentages of fructose and glucose are recorded in Table XXXIV. Glucose accumulated in the series supplied with glu-

TABLE XXXII

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE OR FRUCTOSE WITH AND WITHOUT SODIUM SELENITE (100 P.P.M. Se) FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	66.04±0.024	0.405±0.000	2.978±0.012	3.540±0.012
Glucose	64.11±0.005	1.087±0.020	5.691±0.035	7.079±0.017
Fructose	61.91±0.005	1.383±0.004	4.800±0.002	6.435±0.001
Both	61.77±0.038	1.166±0.010	4.244±0.018	5.633±0.029
Glucose + 100 p.p.m. Se	64.59±0.014	2.846±0.005	2.787±0.006	5.780±0.000
Fructose + 100 p.p.m. Se ...	63.27±0.043	2.222±0.019	3.130±0.010	5.517±0.008
Both + 100 p.p.m. Se	61.94±0.110	2.540±0.016	3.254±0.028	5.966±0.014

TABLE XXXIII

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE OR FRUCTOSE WITH AND WITHOUT SODIUM SELENITE (100 P.P.M. Se) FOR 24 HOURS, CALCULATED FROM TABLE XXXII

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	3.539	2.713	76.66
Fructose	2.895	1.822	62.93
Both	2.093	1.266	60.48
Glucose + Se	2.240	-0.191	0
Fructose + Se	1.977	0.152	7.68
Both + Se	2.426	0.276	11.37

TABLE XXXIV

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE OR FRUCTOSE WITH AND WITHOUT SODIUM SELENITE (100 P.P.M. Se) FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.146±0.029		0.259±0.028	
Glucose	0.193±0.025	0.047	0.894±0.046	0.635
Fructose	0.926±0.025	0.780	0.457±0.021	0.198
Both	0.701±0.021	0.555	0.464±0.031	0.205
Glucose + Se	0.545±0.082	0.399	2.301±0.087	2.042
Fructose + Se	2.060±0.072	1.914	0.177±0.084	-0.082
Both + Se	1.496±0.051	1.350	1.044±0.036	0.785

cose plus selenite, while fructose accumulated in the series supplied with fructose plus selenite.

It is evident that sodium selenite prevented the interconversion of glucose and fructose whichever sugar was supplied. Selenite also prevented the formation of

sucrose whichever sugar was supplied. Some process necessary for synthesis in addition to interconversion of glucose and fructose was also inhibited by selenite, for the synthesis of sucrose was inhibited when both glucose and fructose were supplied.

9. *Effect of arsenite and selenite on photosynthesis and sucrose synthesis:*

A comparison of the effects of arsenite and selenite upon the formation of sucrose in detached blades in the light supplied with water and in the dark supplied with glucose was undertaken, with the results shown in Tables XXXV and XXXVI.

Table XXXV shows that the formation of sucrose was decreased considerably by arsenite and selenite, both in the light and in the dark. Table XXXVI shows that the blades supplied with glucose with arsenite or selenite in the dark accumulated glucose and lost fructose, in agreement with the results presented in Tables XXII, XXV, XXXI, and XXXIV. The blades in water in the light also accumulated glucose but not fructose, when given arsenite or selenite. Since blades known to be supplied with glucose accumulate glucose, and blades known to be supplied with fructose accumulate fructose, in the presence of arsenite or selenite, the results in Table XXXVI constitute strong evidence that the first sugar formed in photosynthesis is glucose alone.

TABLE XXXV

MOISTURE AND SUGAR PERCENTAGES IN BLADES IN THE LIGHT WITH WATER AND IN THE DARK WITH GLUCOSE, WITH AND WITHOUT SODIUM ARSENITE (5-100 P.P.M. As) OR SODIUM SELENITE (5-100 P.P.M. Se) FOR 12 HOURS

Series	Moisture	Reducing sugars	Sucrose	Gain in sucrose	Total sugars
Initial control	71.52±0.057	0.974±0.030	3.552±0.040		4.713±0.011
Water in light	68.11±0.071	1.910±0.017	7.017±0.040	3.465	9.296±0.059
Water + 5 p.p.m. As. . . .	68.40±0.048	1.584±0.020	5.380±0.020	1.828	7.247±0.000
Water + 25 p.p.m. As. . .	69.31±0.119	1.486±0.009	4.604±0.027	1.052	6.332±0.018
Water + 100 p.p.m. As. .	69.59±0.057	1.269±0.042	4.231±0.005	0.679	5.722±0.037
Water + 5 p.p.m. Se. . . .	69.63±0.124	1.503±0.018	5.707±0.021	2.155	7.511±0.004
Water + 25 p.p.m. Se. . .	68.76±0.057	1.424±0.012	5.785±0.021	2.233	7.514±0.035
Water + 100 p.p.m. Se. .	69.48±0.038	1.411±0.007	4.751±0.018	1.199	6.412±0.011
Glucose in dark	70.03±0.052	1.248±0.012	4.453±0.055	0.901	5.935±0.045
Glucose + 5 p.p.m. As. . .	70.05±0.062	1.342±0.003	4.351±0.006	0.799	5.923±0.010
Glucose + 25 p.p.m. As. .	71.15±0.091	1.472±0.003	4.023±0.018	0.471	5.707±0.021
Glucose + 100 p.p.m. As .	70.64±0.000	1.588±0.011	3.373±0.000	-0.179	5.139±0.011
Glucose + 5 p.p.m. Se. . .	69.18±0.029	1.236±0.000	4.610±0.027	1.058	6.089±0.029
Glucose + 25 p.p.m. Se. .	68.83±0.100	1.376±0.005	4.322±0.009	0.770	5.926±0.004
Glucose + 100 p.p.m. Se .	70.30±0.114	1.890±0.000	3.647±0.004	0.095	5.530±0.006

TABLE XXXVI

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES IN THE LIGHT WITH WATER AND IN THE DARK WITH GLUCOSE, WITH AND WITHOUT SODIUM ARSENITE (5-100 P.P.M. As) OR SODIUM SELENITE (5-100 P.P.M. Se), FOR 12 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.798±0.013		0.175±0.043	
Water in light	1.445±0.009	0.647	0.465±0.009	0.290
Water + 5 p.p.m. As	0.968±0.024	0.170	0.616±0.044	0.441
Water + 25 p.p.m. As	0.761±0.018	-0.037	0.725±0.028	0.550
Water + 100 p.p.m. As	0.824±0.002	0.026	0.445±0.045	0.270
Water + 5 p.p.m. Se	0.812±0.050	0.014	0.691±0.032	0.516
Water + 25 p.p.m. Se	0.871±0.014	0.073	0.553±0.027	0.378
Water + 100 p.p.m. Se	0.758±0.011	-0.040	0.653±0.019	0.478
Glucose in dark	0.522±0.025	-0.276	0.726±0.012	0.551
Glucose + 5 p.p.m. As	0.596±0.004	-0.202	0.746±0.000	0.571
Glucose + 25 p.p.m. As	0.574±0.013	-0.224	0.898±0.010	0.723
Glucose + 100 p.p.m. As	0.544±0.016	-0.254	1.044±0.005	0.869
Glucose + 5 p.p.m. Se	0.662±0.039	-0.136	0.574±0.039	0.399
Glucose + 25 p.p.m. Se	0.412±0.011	-0.386	0.964±0.017	0.789
Glucose + 100 p.p.m. Se	0.295±0.014	-0.503	1.395±0.013	1.220

10. Fluoride:

Fluoride exerts a depressing effect upon several steps in respiratory and fermentative processes. The effects of fluoride were summarized by Elvehjem (23), who stated that fluoride partially inhibits succinic acid dehydrogenase and alphaphosphoglycerol dehydrogenase. Fluoride also inhibits lactic dehydrogenase but not malic dehydrogenase. Fluoride inhibits glycolysis chiefly by inhibiting the splitting of phosphate esters. Sodium fluoride (0.005 M) inhibits the rephosphorylation of adenylic acid by intermediate products of glycogenolysis. According to MacFarlane (55), 0.02 M sodium fluoride is required for 100 per cent inhibition of the breakdown of phosphoglyceric acid to phosphopyruvic acid. King (46) stated that the enzymic synthesis of cocarboxylase is inhibited by fluoride. Borei (5) stated that fluoride had no effect upon cytochrome C or upon cytochrome oxidase. Runnström, Borei, and Sperber (74) reported that fluoride depressed the respiration of yeast by its effect on an intermediate substance between the dehydrogenase system and the cytochrome system. This is considered by Szent-Györgyi (79) to be the point where fumaric, succinic, and oxaloacetic acids enter into the respiratory scheme. Kalckar (42) found that esterification of glucose with inorganic phosphate in cell-free kidney extract occurred under aerobic conditions, and that a mixture of fructose diphosphate and phosphoglyceric acid accumulated in the presence of sodium fluoride. Case (8) reported that hexokinase is inhibited by M/50 sodium fluoride (= 0.02 M), while Iri (40) found that 0.01 M fluoride had no effect upon hexokinase.

Several tests have been conducted to find the effect of various concentrations of fluoride upon interconversion and synthesis. The results of an experiment using glucose are presented in Table XXXVII. The gains in sugars and the synthetic efficiencies are shown in Table XXXVIII. The percentages of fructose and glucose

are reported in Table XXXIX. It is evident that fluoride exerted a depressing effect both upon the conversion of glucose to fructose and upon the formation of sucrose.

TABLE XXXVII

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT SODIUM FLUORIDE (47 P.P.M. F-380 P.P.M. F) FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	71.74±0.167	0.898±0.011	1.607±0.023	2.590±0.013
Glucose	70.08±0.114	1.387±0.009	5.965±0.048	7.666±0.060
Glucose + 47 p.p.m. F	70.54±0.162	1.477±0.005	5.651±0.004	7.426±0.000
Glucose + 95 p.p.m. F	71.19±0.052	1.693±0.001	4.914±0.002	6.866±0.003
Glucose + 190 p.p.m. F	70.88±0.086	2.745±0.009	4.072±0.003	7.031±0.013
Glucose + 380 p.p.m. F	70.29±0.119	3.931±0.013	3.089	7.212

TABLE XXXVIII

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT SODIUM FLUORIDE (47 P.P.M.-380 P.P.M. F) FOR 24 HOURS, CALCULATED FROM TABLE XXXVII

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	5.076	4.358	85.85
Glucose + 47 p.p.m. F	4.836	4.044	83.62
Glucose + 95 p.p.m. F	4.276	3.307	77.33
Glucose + 190 p.p.m. F	4.441	2.465	55.50
Glucose + 380 p.p.m. F	4.622	1.482	32.06

TABLE XXXIX

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT SODIUM FLUORIDE (47 P.P.M.-380 P.P.M. F) FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.589±0.024		0.308±0.035	
Glucose	0.695±0.005	0.106	0.691±0.004	0.383
Glucose + 47 p.p.m. F	0.639±0.018	0.050	0.838±0.013	0.530
Glucose + 95 p.p.m. F	0.562±0.021	-0.027	1.131±0.023	0.823
Glucose + 190 p.p.m. F	0.930±0.046	0.341	1.814±0.056	1.506
Glucose + 380 p.p.m. F	0.564±0.020	-0.025	3.367±0.034	3.059

Another experiment was conducted in which both glucose and fructose were used, and the results are set out in Table XL. The gains in sugars and the synthetic efficiencies are reported in Table XLI. The percentages of fructose and glucose are presented in Table XLII. Fluoride exerted a depressing effect upon the intercon-

TABLE XL

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE OR FRUCTOSE WITH AND WITHOUT SODIUM FLUORIDE (95 P.P.M. F-380 P.P.M. F) FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	72.43±0.014	0.844±0.002	1.652±0.021	2.583±0.025
Glucose	72.91±0.038	1.076±0.008	3.454±0.012	4.713±0.022
Fructose	72.56±0.043	1.017±0.017	3.527±0.031	4.729±0.050
Both	71.54±0.052	0.883±0.003	3.433±0.046	4.497±0.051
Glucose + 95 p.p.m. F	72.06±0.029	1.225±0.007	2.800±0.013	4.173±0.021
Fructose + 95 p.p.m. F	72.37±0.005	1.379±0.001	2.853±0.007	4.382±0.009
Both + 95 p.p.m. F	71.82±0.029	1.298±0.002	2.944±0.004	4.397±0.006

Glucose + 190 p.p.m. F	71.87±0.052	1.376±0.014	2.639±0.026	4.155±0.013
Fructose + 190 p.p.m. F	71.39±0.052	1.486±0.000	2.856±0.002	4.493±0.002
Both + 190 p.p.m. F	71.42±0.052	1.562±0.011	3.047±0.031	4.770±0.021
Glucose + 380 p.p.m. F	71.52±0.000	2.016±0.014	2.781±0.000	4.944±0.015
Fructose + 380 p.p.m. F	71.78±0.029	1.892±0.004	2.866±0.005	4.909±0.009
Both + 380 p.p.m. F	71.58±0.029	2.033±0.008	2.711±0.008	4.887±0.000

version of glucose and fructose and the formation of sucrose whichever sugar was supplied. The depressing effect was correlated with the amount of fluoride supplied. Since the synthetic efficiency was depressed just as much when both glucose and fructose were supplied as when either alone was given to the blades, fluoride must affect some process necessary for synthesis in addition to its effect upon interconversion of glucose and fructose. However, even 380 p.p.m. F (= 0.02 M) did not inhibit synthesis completely.

TABLE XLI

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE OR FRUCTOSE WITH AND WITHOUT SODIUM FLUORIDE (95 P.P.M.-380 P.P.M. F) FOR 24 HOURS, CALCULATED FROM TABLE XL

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	2.130	1.802	84.60
Fructose	2.146	1.875	87.37
Both	1.914	1.781	93.05
Glucose + 95 p.p.m. F	1.590	1.148	72.20
Fructose + 95 p.p.m. F	1.799	1.201	66.75
Both + 95 p.p.m. F	1.814	1.292	71.22
Glucose + 190 p.p.m. F	1.572	0.987	62.78
Fructose + 190 p.p.m. F	1.910	1.204	63.03
Both + 190 p.p.m. F	2.187	1.395	63.78
Glucose + 380 p.p.m. F	2.361	1.129	47.81
Fructose + 380 p.p.m. F	2.326	1.214	52.19
Both + 380 p.p.m. F	2.304	1.059	45.96

TABLE XLII

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE OR FRUCTOSE WITH AND WITHOUT SODIUM FLUORIDE (95 P.P.M. F-380 P.P.M. F) FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.824±0.006		0.019±0.004	
Glucose	0.592±0.033	-0.232	0.484±0.042	0.465
Fructose	0.921±0.049	0.097	0.096±0.032	0.077
Both	0.437±0.007	-0.387	0.446±0.009	0.427
Glucose + 95 p.p.m. F	0.812±0.022	-0.012	0.413±0.029	0.394
Fructose + 95 p.p.m. F	1.289±0.044	0.465	0.090±0.043	0.071
Both + 95 p.p.m. F	0.849±0.019	0.025	0.449±0.017	0.430
Glucose + 190 p.p.m. F	0.782±0.007	-0.042	0.594±0.007	0.575
Fructose + 190 p.p.m. F	1.346±0.014	0.522	0.140±0.014	0.121
Both + 190 p.p.m. F	0.960±0.041	0.136	0.602±0.029	0.583
Glucose + 380 p.p.m. F	0.614±0.016	-0.210	1.402±0.030	1.383
Fructose + 380 p.p.m. F	1.866±0.022	1.042	0.032±0.015	0.013
Both + 380 p.p.m. F	1.350±0.039	0.526	0.682±0.031	0.663

11. Malonate:

Malonate inhibits succinic dehydrogenase, but in the presence of fumaric acid, tissues are insensitive to malonate, according to Oppenheimer (67). Elvehjem (23) stated that malonate inhibits succinic dehydrogenase, lactic dehydrogenase, the oxidation of fatty acids, glucose oxidation by brain, and the oxidation of ethyl alcohol by liver.

A test was conducted to find the effect of malonate with and without fumaric acid upon interconversion and synthesis. The results are presented in Table XLIII. The gains in sugars and the synthetic efficiencies are shown in Table XLIV. The percentages of fructose and glucose are presented in Table XLV. It is true that

TABLE XLIII

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT SODIUM MALONATE (0.5%) AND FUMARIC ACID (0.4%) FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	67.92±0.029	0.338±0.001	2.507±0.013	2.978±0.016
Glucose	65.84±0.029	1.285±0.001	4.818±0.046	6.358±0.047
Glucose + fumaric acid	66.95±0.052	1.272±0.000	3.921±0.027	5.399±0.030
Glucose + malonate	67.26±0.000	0.963±0.017	3.453±0.020	4.599±0.005
Glucose + malonate + fumaric acid	66.08±0.033	1.101±0.000	4.218±0.004	5.541±0.005

TABLE XLIV

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT SODIUM MALONATE (0.5%) AND FUMARIC ACID (0.4%) FOR 24 HOURS, CALCULATED FROM TABLE XLIII

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	3.380	2.311	68.37
Glucose + fumaric acid	2.421	1.414	58.40
Glucose + malonate	1.621	0.946	58.35
Glucose + malonate + fumaric acid .	2.563	1.711	66.75

TABLE XLV

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT SODIUM MALONATE (0.5%) AND FUMARIC ACID (0.4%) FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.374±0.010		0 ±0.000	
Glucose	0.563±0.031	0.189	0.722±0.030	0.722
Glucose + fumaric acid	0.819±0.051	0.445	0.453±0.050	0.453
Glucose + malonate	0.783±0.049	0.409	0.180±0.066	0.180
Glucose + malonate + fumaric acid	0.483±0.003	0.109	0.618±0.004	0.618

sodium malonate decreased the synthetic efficiency when used alone but not when used with fumaric acid which would be expected if the effect is on succinic dehydrogenase. However, the depressing effect was so little compared with the effects of arsenite, selenite, and fluoride, and considering the comparatively strong concentration of sodium malonate used, that there is no real evidence of inhibition by malonate.

Neither was there any evidence of an inhibitory effect of malonate upon the conversion of glucose to fructose.

12. *Acenaphthene*:

Shmuck (75) found that acenaphthene interfered with the metabolism of wheat sprouts by reducing respiration, acidifying the sap presumably through the formation of free phosphoric acid from nucleoproteins, and doubling the glucolytic action.

Acenaphthene was dissolved in hot alcohol and diluted with glucose solution, resulting in a milky liquid. An equal amount of alcohol was used in the glucose control. The results of the test are presented in Table XLVI. The gains in sugars and the synthetic efficiencies are reported in Table XLVII. The percentages of fructose and glucose are recorded in Table XLVIII. Acenaphthene appeared to aid both the conversion of glucose to fructose and the formation of sucrose a little.

TABLE XLVI

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT ACENAPHTHENE (0.1%) FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	71.38±0.014	1.088±0.012	2.263±0.003	3.470±0.009
Glucose	70.62±0.052	1.785±0.006	3.952±0.009	5.945±0.003
Glucose + acenaphthene	70.21±0.009	2.012±0.014	4.949±0.019	7.222±0.034

TABLE XLVII

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE, WITH AND WITHOUT ACENAPHTHENE (0.1%) FOR 24 HOURS, CALCULATED FROM TABLE XLVI

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	2.475	1.689	68.24
Glucose + acenaphthene	3.752	2.686	71.58

TABLE XLVIII

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT ACENAPHTHENE (0.1%) FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.743±0.001		0.345±0.010	
Glucose	0.640±0.019	-0.103	1.145±0.013	0.800
Glucose + acenaphthene	1.333±0.009	0.590	0.679±0.004	0.334

13. *Chloroform*:

Irving (41) reported that small doses of chloroform increased the intensity of respiration in cherry laurel leaves, medium doses caused an initial increase followed by a decrease to below normal, and strong doses gave a rapid fall to zero. Deleano and Dick (17) found that translocation of material from the leaves of *Vitis vinifera* was not affected by chloroforming the petioles, and that chloroform had no effect upon enzyme activity and starch hydrolysis. Spoehr and Milner (77), however, found that leaves of *Helianthus annuus* and *Nicotiana Tabacum* when killed by means of chloroform retained their amylase but showed no starch dissolution even after 143 days, and suggested that the treatment with chloroform resulted in marked

changes in the finer structure of the cell and in colloidal phase relationships of the components of the chloroplasts and the surrounding protoplasm.

Blades of sugar cane were placed under a bell jar with chloroform for two hours. For the first hour the chloroform was in a beaker, but for the second hour cotton soaked in chloroform was used. The leaves did not become flaccid but they did become very cold. After the two hours' exposure to chloroform, the leaves were supplied with glucose for 24 hours. The results are set out in Table XLIX. The gains in sugars and the synthetic efficiencies are presented in Table L. The percentages of fructose and glucose are reported in Table LI. The treatment with chloroform decreased the synthetic efficiency considerably, but did not inhibit synthesis entirely. The conversion of glucose to fructose was also diminished by the treatment with chloroform.

TABLE XLIX

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE FOR 24 HOURS, WITH AND WITHOUT PREVIOUS EXPOSURE TO CHLOROFORM FOR 2 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	66.06±0.024	0.550±0.002	1.802±0.008	2.447±0.007
Glucose	67.23±0.105	2.070±0.034	5.546±0.017	7.909±0.003
Chloroform-glucose	67.74±0.000	2.941±0.015	3.862±0.012	7.006±0.027

TABLE L

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE FOR 24 HOURS, WITH AND WITHOUT PREVIOUS EXPOSURE TO CHLOROFORM FOR 2 HOURS, CALCULATED FROM TABLE XLIX

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	5.462	3.744	68.54
Chloroform-glucose	4.559	2.060	45.18

TABLE LI

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE FOR 24 HOURS, WITH AND WITHOUT PREVIOUS EXPOSURE TO CHLOROFORM FOR 2 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.672±0.023		0 ±0.000	
Glucose	0.737±0.029	0.065	1.334±0.008	1.334
Chloroform-glucose	0.689±0.023	0.017	2.251±0.038	2.251

14. Dinitrophenol:

Norris (64) reported that 2:4-dinitrophenol caused an increase in oxygen consumption. du Buy and Olson (21) found that dinitrophenol (50 p.p.m. for 30 minutes) markedly inhibited the respiration of *Avena* coleoptile, and that respiration was completely stopped by 100 p.p.m. for 30 minutes. Oppenheimer (67) stated that dinitrophenol increased the rate of oxidation in animals and plants, and that while it increased both respiration and glucolysis, the main effect was a stimulation of anaerobic sugar breakdown.

The effect of dinitrophenol upon the transformations of sugars was studied, using 5, 50, and 100 p.p.m. The results for moisture and sugars are recorded in Table

LII. The gains in sugars and the synthetic efficiencies are reported in Table LIII. Dinitrophenol appeared to diminish synthesis a little, in the higher concentrations.

TABLE LII

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT 2:4-DINITROPHENOL (5 P.P.M.-100 P.P.M.) FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	68.23 \pm 0.095	0.752 \pm 0.028	2.277 \pm 0.003	3.150 \pm 0.025
Glucose	68.58 \pm 0.009	1.273 \pm 0.019	4.835 \pm 0.030	6.363 \pm 0.050
Glucose + 5 p.p.m. DNP	68.83 \pm 0.028	1.209 \pm 0.006	4.655 \pm 0.005	6.110 \pm 0.000
Glucose + 50 p.p.m. DNP ...	69.70 \pm 0.019	1.413 \pm 0.008	4.584 \pm 0.041	6.239 \pm 0.035
Glucose + 100 p.p.m. DNP ..	69.00 \pm 0.133	1.504 \pm 0.012	4.686 \pm 0.018	6.437 \pm 0.030

TABLE LIII

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT 2:4-DINITROPHENOL (5 P.P.M.-100 P.P.M.) FOR 24 HOURS, CALCULATED FROM TABLE LII

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	3.213	2.558	79.61
Glucose + 5 p.p.m. DNP	2.960	2.378	80.33
Glucose + 50 p.p.m. DNP	3.089	2.307	74.68
Glucose + 100 p.p.m. DNP	3.287	2.409	73.28

15. Ethyl alcohol:

Elvehjem stated that ethyl alcohol completely stopped the reduction of cytochrome.

The effect of 2 per cent ethyl alcohol upon synthesis is shown in Tables LIV and LV. The effect of 5 per cent ethyl alcohol was then studied, with the results reported in Tables LVI and LVII. In both of these tests ethyl alcohol appeared to raise the synthetic efficiency a little.

TABLE LIV

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT 2% ETHYL ALCOHOL FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	73.07 \pm 0.033	0.827 \pm 0.002	2.301 \pm 0.022	3.250 \pm 0.025
Glucose	72.59	2.105 \pm 0.002	7.282 \pm 0.014	9.771 \pm 0.012
Glucose + alcohol	71.79 \pm 0.076	1.850 \pm 0.011	7.380 \pm 0.001	9.619 \pm 0.009

TABLE LV

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT 2% ETHYL ALCOHOL FOR 24 HOURS, CALCULATED FROM TABLE LIV

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	6.521	4.981	76.38
Glucose + alcohol	6.369	5.079	79.74

TABLE LVI

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT 5% ETHYL ALCOHOL FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	71.39±0.019	1.495±0.009	3.047±0.005	4.703±0.003
Water	73.08±0.048	1.683±0.013	2.412±0.009	4.222±0.023
Alcohol	72.30±0.033	1.254±0.006	2.345±0.018	3.723±0.026
Glucose	72.01±0.052	1.960±0.013	4.894±0.022	7.112±0.010
Glucose + alcohol	71.35±0.095	1.970±0.003	5.078±0.017	7.310±0.019

TABLE LVII

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT 5% ETHYL ALCOHOL FOR 24 HOURS, CALCULATED FROM TABLE LV

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Water	-0.481	-0.635	
Alcohol	-0.980	-0.702	
Glucose	2.409	1.847	76.67
Glucose + alcohol	2.607	2.031	77.90

16. Histidine:

Norris (64) reported that histidine, in 6×10^{-7} molar concentration, reduced respiration.

The effect of histidine upon synthesis is shown in Tables LVIII and LVIX. The fructose and glucose percentages are presented in Table LX. Histidine ap-

TABLE LVIII

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT HISTIDINE FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	68.21±0.076	0.815±0.003	2.151±0.003	3.080±0.007
Glucose	69.98±0.024	1.330±0.006	4.659±0.008	6.234±0.003
Glucose + histidine 6×10^{-7} M	69.65±0.009	1.243±0.007	4.548±0.000	6.031±0.008
Glucose + histidine 6×10^{-6} M	70.07±0.067	1.269±0.002	4.738±0.000	6.256±0.003
Glucose + histidine 6×10^{-5} M	68.55±0.086	1.165±0.007	4.726±0.008	6.141±0.016
Glucose + histidine 6×10^{-4} M	67.63±0.043	0.957±0.004	4.095±0.005	5.268±0.000

TABLE LIX

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT HISTIDINE FOR 24 HOURS, CALCULATED FROM TABLE LVIII

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	3.154	2.508	79.51
Glucose + histidine 6×10^{-7} M	2.951	2.397	81.22
Glucose + histidine 6×10^{-6} M	3.176	2.587	81.45
Glucose + histidine 6×10^{-5} M	3.061	2.575	84.12
Glucose + histidine 6×10^{-4} M	2.188	1.944	88.84

TABLE LX

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT HISTIDINE FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.459±0.011		0.356±0.014	
Glucose	0.921±0.031	0.462	0.409±0.037	0.053
Glucose + histidine 6x10 ⁻⁷ M.....	0.919±0.034	0.460	0.324±0.041	-0.032
Glucose + histidine 6x10 ⁻⁶ M.....	0.831±0.045	0.372	0.437±0.043	0.081
Glucose + histidine 6x10 ⁻⁵ M.....	0.891±0.041	0.432	0.274±0.049	-0.082
Glucose + histidine 6x10 ⁻⁴ M.....	0.934±0.014	0.475	0.030±0.014	-0.326

peared to increase synthesis a little, particularly in 6x10⁻⁴ M concentration. There was no apparent effect upon the conversion of glucose to fructose.

17. Phloridzin:

According to Elvehjem, 0.005–0.2 M phloridzin inhibited phosphorylation and dephosphorylation in yeast and muscle. It also inhibited the formation of lactic acid from glucose or glycogen. 0.01 M phloridzin inhibited the dismutation of triose phosphate to phosphoglyceric and glycerophosphoric acids in muscle. In dried yeast, phloridzin almost completely inhibited the formation of adenosine triphosphate from adenosine phosphate and fructose diphosphate. Ostern, Baranowski and Terszakowec' (68) reported that phloridzin, 7x10⁻³ M, gave 100 per cent inhibition of adenosine phosphorylase. Dahl (14) found that phloridzin hindered the phosphorylation of starch and glycogen in muscle extract. Cori and Cori (12) state that phloridzin inhibited the action of phosphorylase, which catalyzes the reaction glycogen + inorganic phosphate ⇌ glucose-1-phosphate, in both directions. Lisitsyn (54) found that 0.005 M phloridzin inhibited the synthesis of sucrose in the leaves of *Arctium lappa* and *Crataegus* sp. but not in the leaves of *Tussilago farfara*.

The effect of phloridzin upon sugar transformations is shown in Table LXI. The gains in sugars and the synthetic efficiencies are reported in Table LXII. The percentages of fructose and glucose are recorded in Table LXIII. Phloridzin did not inhibit either the interconversion of glucose and fructose or the synthesis of sucrose.

TABLE LXI

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE OR FRUCTOSE WITH AND WITHOUT PHLORIDZIN (0.01 M) FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	71.44±0.033	0.902±0.001	2.293±0.000	3.316±0.000
Glucose	71.04±0.067	1.225±0.004	4.771±0.016	6.247±0.020
Fructose	72.24±0.019	1.395±0.003	4.656±0.000	6.297±0.004
Both	71.91±0.019	1.283±0.048	4.748±0.021	6.281±0.027
Glucose + phloridzin	71.56±0.038	1.135±0.004	4.440±0.000	5.854±0.017
Fructose + phloridzin	71.79±0.009	1.418±0.009	4.381±0.014	6.030±0.005
Both + phloridzin	71.33±0.043	1.168±0.012	4.405±0.033	5.805±0.022

TABLE LXII

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE OR FRUCTOSE WITH AND WITHOUT PHLORIDZIN (0.01 M) FOR 24 HOURS, CALCULATED FROM TABLE LXI

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	2.931	2.478	84.54
Fructose	2.981	2.363	79.26
Both	2.965	2.455	82.79
Glucose + phloridzin	2.538	2.147	84.59
Fructose + phloridzin	2.714	2.088	76.93
Both + phloridzin	2.489	2.112	84.85

TABLE LXIII

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE OR FRUCTOSE WITH AND WITHOUT PHLORIDZIN (0.01 M) FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.512 ± 0.016		0.390 ± 0.017	
Glucose	0.648 ± 0.021	0.136	0.576 ± 0.025	0.186
Fructose	1.289 ± 0.009	0.777	0.106 ± 0.013	-0.284
Both	1.136 ± 0.015	0.624	0.147 ± 0.019	-0.243
Glucose + phloridzin	0.667 ± 0.031	0.155	0.468 ± 0.027	0.078
Fructose + phloridzin	1.190 ± 0.026	0.678	0.228 ± 0.035	-0.162
Both + phloridzin	1.015 ± 0.004	0.503	0.153 ± 0.017	-0.237

18. Quinine:

Enders and Wieninger (24) reported that quinine and several other alkaloids inhibited growth and fermentation by yeast. Fermentation was inhibited by lower concentrations than were required to inhibit multiplication.

The effect of quinine (0.01%) upon the formation of sucrose was tried in two tests. In the first test the synthetic efficiencies were as follows: with glucose 86.56; with glucose + quinine, 93.87. In the second test the synthetic efficiencies were as follows: with glucose, 82.04; with glucose + quinine, 82.20; with fructose, 78.40; with fructose + quinine, 87.95; with glucose and fructose, 89.94; with glucose and fructose + quinine, 89.09. It is evident that the effect of quinine needs further study before drawing any conclusion.

19. Urethane:

Elvehjem stated that urethane completely stopped the reduction of cytochrome. It partially inhibited alpha-phosphoglycerol dehydrogenase. In brain, urethane was found to inhibit the oxidation of glucose, lactate, and pyruvate but not of succinate. Kempner (45) stated that if a respiratory process is not affected by narcotics, that process is not bound to cellular structure. Burris and Wilson (7) stated that urethane, like other indifferent narcotics, is considered to be a general inhibitor of dehydrogenases and to exert its action by being adsorbed on enzyme surfaces.

Urethane was used in a single test with blades of sugar cane supplied with glucose, with the results presented in Table LXIV. The gains in sugars and the synthetic efficiencies are recorded in Table LXV. The percentages of fructose and glu-

TABLE LXIV

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT URETHANE (1%) FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	69.26±0.048	0.719±0.001	2.669±0.008	3.528±0.009
Glucose	69.39±0.029	1.438±0.030	6.259±0.009	8.027±0.040
Glucose + urethane	68.56±0.129	1.342±0.003	5.794±0.018	7.441±0.015

TABLE LXV

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT URETHANE (1%) FOR 24 HOURS, CALCULATED FROM TABLE LXIV

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	4.499	3.590	79.79
Glucose + urethane	3.913	3.125	79.86

cose are reported in Table LXVI. Urethane appeared to decrease the absorption of sugar a little, as shown by the effect on the gain in total sugars. There was no effect upon synthesis or upon the conversion of glucose to fructose.

TABLE LXVI

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT URETHANE (1%) FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.279±0.045		0.440±0.043	
Glucose	0.796±0.020	0.517	0.642±0.009	0.202
Glucose + urethane	0.714±0.019	0.435	0.628±0.016	0.188

20. Brilliant alizarine blue and rosinduline GG:

Michaelis and Smythe (61) studied the effects of several dyes upon fermentation in yeast, and reported that some dyes prevented the enzymatic conversion of fructose monophosphate to fructose diphosphate. The dyes with the strongest inhibitory effect were brilliant alizarine blue and rosinduline GG. The concentration employed was 4.9×10^{-3} M. These dyes were used by Smythe (76) for the quantitative production of the hexose monophosphates.

Experiments have been conducted with both of these dyes to find their effects upon interconversion and synthesis in the sugar cane plant. The results of a test with brilliant alizarine blue are presented in Table LXVII. The gains in sugars and the synthetic efficiencies are recorded in Table LXVIII. The percentages of

TABLE LXVII

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE OR FRUCTOSE WITH AND WITHOUT BRILLIANT ALIZARINE BLUE (4.9×10^{-3} M) FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	65.90±0.119	0.431±0.001	2.653±0.005	3.224±0.004
Glucose	66.19±0.000	1.489±0.004	5.149±0.030	6.910±0.028
Fructose	65.45±0.024	1.134±0.013	4.644±0.041	6.022±0.029
Glucose + BAB	61.26±0.076	1.077±0.005	2.866±0.013	4.094±0.019
Fructose + BAB	57.17±0.024	1.214±0.122	2.526±0.023	3.874±0.097

TABLE LXVIII

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE OR FRUCTOSE WITH AND WITHOUT BRILLIANT ALIZARINE BLUE (4.9×10^{-3} M) FOR 24 HOURS, CALCULATED FROM TABLE LXVII

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	3.686	2.496	67.71
Fructose	2.798	1.991	71.15
Glucose + BAB	0.870	0.213	24.48
Fructose + BAB	0.650	-0.127	0

fructose and glucose are reported in Table LXIX. Brilliant alizarine blue decreased the absorption of sugar and inhibited the synthesis of sucrose, but did not inhibit the conversion of fructose to glucose, although it may have decreased the conversion of glucose to fructose. In another test with the same concentration of brilliant alizarine blue, the synthetic efficiencies were as follows: glucose, 68.25; glucose + BAB, 0; fructose, 61.07; fructose + BAB, 17.26. The dye inhibited the formation of sucrose from either glucose or fructose.

TABLE LXIX

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE OR FRUCTOSE WITH AND WITHOUT BRILLIANT ALIZARINE BLUE (4.9×10^{-3} M) FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.426 ± 0.000		0.005 ± 0.002	
Glucose	0.418 ± 0.009	-0.008	1.071 ± 0.013	1.066
Fructose	0.743 ± 0.039	0.317	0.390 ± 0.026	0.385
Glucose + BAB	0.440 ± 0.048	0.014	0.637 ± 0.053	0.632
Fructose + BAB	0.456 ± 0.024	0.030	0.758 ± 0.098	0.753

Rosinduline GG was used in one test with sugar cane. The concentration used by Michaelis and Smythe was 5×10^{-3} M. I could not find the formula or the molecular weight of rosinduline GG; the concentration used was 1.87 grams per liter. The results are set out in Table LXX. The gains in sugars and the synthetic efficiencies are reported in Table LXXI. The percentages of fructose and glucose are recorded in Table LXXII. The formation of sucrose was cut in half by the use

TABLE LXX

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE OR FRUCTOSE WITH AND WITHOUT ROSINDULINE GG (1.87 GM/L) FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	65.94 ± 0.043	0.787 ± 0.007	4.172 ± 0.003	5.179 ± 0.010
Glucose	66.69 ± 0.014	0.995 ± 0.020	6.585 ± 0.049	7.926 ± 0.031
Fructose	66.05 ± 0.081	1.132 ± 0.022	7.160 ± 0.027	8.669 ± 0.006
Both	68.21 ± 0.013	1.302 ± 0.025	6.973 ± 0.030	8.642 ± 0.006
Glucose + ros. GG	63.47 ± 0.071	2.075 ± 0.009	5.063 ± 0.000	7.405 ± 0.009
Fructose + ros. GG	63.95 ± 0.005	2.104 ± 0.115	4.890 ± 0.208	7.251 ± 0.334
Both + ros. GG	62.43 ± 0.057	1.920 ± 0.028	5.626 ± 0.027	7.842 ± 0.000

TABLE LXXI

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE OR FRUCTOSE WITH AND WITHOUT ROSINDULINE GG (1.87 GM/1) FOR 24 HOURS, CALCULATED FROM TABLE LXX

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	2.747	2.413	87.84
Fructose	3.490	2.988	85.61
Both	3.463	2.801	80.88
Glucose + ros. GG	2.226	0.891	40.02
Fructose + ros. GG	2.072	0.718	34.65
Both + ros. GG	2.663	1.454	54.60

TABLE LXXII

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE OR FRUCTOSE WITH AND WITHOUT ROSINDULINE GG (1.87 GM/1) FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.658±0.043		0.129±0.050	
Glucose	0.778±0.005	0.120	0.216±0.026	0.087
Fructose	1.112±0.029	0.454	0.064±0.030	-0.065
Both	1.432±0.021	0.774	0.000±0.000	-0.129
Glucose + ros. GG	0.734±0.084	0.076	1.341±0.074	1.212
Fructose + ros. GG	1.052±0.053	0.394	1.051±0.061	0.922
Both + ros. GG	0.965±0.029	0.307	0.954±0.000	0.825

of rosinduline GG. More glucose than fructose accumulated whichever sugar was supplied, which indicates that the dye had no deleterious effect upon the conversion of fructose to glucose, but may have decreased the conversion of glucose to fructose.

Since brilliant alizarine blue and rosinduline GG inhibit the formation of fructose diphosphate from fructose monophosphate, according to Michaelis and Smythe, and since these dyes decreased or inhibited the formation of sucrose from glucose, we may conclude either that the dyes exert a specific effect upon synthesis or that the phosphorylation of glucose or fructose must proceed to the fructose diphosphate stage for the synthesis of sucrose to take place.

21. Iodine, silver nitrate, and copper sulphate:

Herbert (37) found that M/50,000 iodine, silver nitrate, or copper sulphate gave 100 per cent inhibition of zymohexase activity in rabbit skeletal muscle. Zymohexase is the enzyme which catalyzes the breakdown of fructose diphosphate forming dihydroxyacetone phosphate and glyceraldehyde phosphate, or triose phosphate. It is found in plants as well as in animals, as Allen (1) stated that the potato contains zymohexase similar to that in muscle. Rapkine (73) reported that triose phosphate dehydrogenase of muscle is inactivated by iodine.

The studies with brilliant alizarine blue and rosinduline GG reported in this paper led to the conclusion that phosphorylation of glucose or fructose must proceed to the fructose diphosphate stage in order for the synthesis of sucrose to take place. The tests with iodoacetate indicated that phosphorylation does not need to proceed beyond the triose phosphate stage for the synthesis of sucrose to occur. To determine whether fructose diphosphate or triose phosphate is the necessary intermediate

for the formation of sucrose, a test was conducted using iodine, silver nitrate, and copper sulphate in the same concentration used by Herbert. If these chemicals should inhibit synthesis then triose phosphate may be the required substrate rather than fructose diphosphate.

The results for moisture and sugars, obtained in the test with iodine, copper sulphate, and silver nitrate, are presented in Table LXXIII. The gains in sugars and the synthetic efficiencies are reported in Table LXXIV. The percentages of fructose and glucose are recorded in Table LXXV.

TABLE LXXIII

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT I_2 , $AgNO_3$, OR $CuSO_4$ (M/50,000) FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	63.20 ± 0.067	0.726 ± 0.005	3.148 ± 0.014	4.040 ± 0.020
Glucose	63.28 ± 0.019	1.186 ± 0.036	5.429 ± 0.017	6.901 ± 0.018
Glucose + I_2	63.55 ± 0.091	1.201 ± 0.000	6.134 ± 0.012	7.658 ± 0.013
Glucose + $AgNO_3$	63.54 ± 0.129	1.073 ± 0.003	5.611 ± 0.028	6.980 ± 0.032
Glucose + $CuSO_4$	62.95 ± 0.033	1.106 ± 0.004	6.234 ± 0.006	7.668 ± 0.010

TABLE LXXIV

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT I_2 , $AgNO_3$, OR $CuSO_4$ (M/50,000) FOR 24 HOURS, CALCULATED FROM TABLE LXXIII

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	2.861	2.281	79.72
Glucose + I_2	3.618	2.986	82.53
Glucose + $AgNO_3$	2.940	2.463	83.77
Glucose + $CuSO_4$	3.628	3.086	85.06

Far from exerting a depressing effect, iodine, silver nitrate, and copper sulphate all increased the synthetic efficiency, according to Table LXXIV. Neither iodine nor copper sulphate affected the conversion of glucose to fructose significantly, but the effect of silver nitrate in depressing the conversion of glucose to fructose may

TABLE LXXV

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT I_2 , $AgNO_3$, OR $CuSO_4$ (M/50,000) FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.484 ± 0.013		0.242 ± 0.019	
Glucose	0.608 ± 0.011	0.124	0.578 ± 0.047	0.336
Glucose + I_2	0.678 ± 0.022	0.194	0.523 ± 0.020	0.281
Glucose + $AgNO_3$	0.102 ± 0.001	-0.382	0.971 ± 0.001	0.729
Glucose + $CuSO_4$	0.402 ± 0.035	-0.082	0.704 ± 0.039	0.462

have been significant. Since inhibiting the breakdown of fructose diphosphate by zymohexase resulted in an increased synthesis of sucrose, the theory is suggested that fructose diphosphate is a necessary stepping stone in the formation of sucrose from glucose.

22. Sodium diethyldithiocarbamate:

Keilin and Hartree (44) found that sodium diethyldithiocarbamate inhibits succinic dehydrogenase one hundred per cent in 2×10^{-5} M concentration. This concen-

tration was used in a test with blades supplied with glucose for 24 hours. The synthetic efficiency in the series with glucose was 78.64, and in the series with glucose plus sodium diethyldithiocarbamate the synthetic efficiency was 75.80.

Since synthesis was depressed so little by the carbamate, this may indicate that succinic dehydrogenase does not take part in the synthesis of sucrose.

23. *Potassium ferricyanide:*

Mendel and Strelitz (59) reported that potassium ferricyanide increased the Pasteur effect, using 10^{-2} mol./liter. Potassium ferricyanide is a mild oxidizing agent.

One test was conducted in which blades were supplied with potassium ferricyanide along with glucose, the results of which are recorded in Table LXXVI. The

TABLE LXXVI

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT POTASSIUM FERRICYANIDE (3.292 GM/1) FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	67.59±0.033	0.694±0.000	2.675±0.011	3.510±0.012
Glucose	64.10±0.014	3.106±0.016	6.793±0.009	10.257±0.006
Glucose + ferricyanide	63.38±0.038	4.765±0.003	5.141±0.029	10.177±0.028

gains in sugars and the synthetic efficiencies are reported in Table LXXVII. The percentages of fructose and glucose are set out in Table LXXVIII. Potassium ferricyanide considerably decreased the synthetic efficiency and caused an accumulation of glucose but not fructose, which indicates that it interfered with the conversion of glucose to fructose and with the synthesis of sucrose.

TABLE LXXVII

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT POTASSIUM FERRICYANIDE (3.292 GM/1) FOR 24 HOURS, CALCULATED FROM TABLE LXXVI

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	6.747	4.118	61.03
Glucose + K ferricyanide	6.667	2.466	36.98

TABLE LXXVIII

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT POTASSIUM FERRICYANIDE (3.292 GM/1) FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.230±0.006		0.464±0.006	
Glucose	0.710±0.040	0.480	2.395±0.057	1.931
Glucose + K ferricyanide	0.730±0.011	0.500	4.034±0.014	3.570

24. *Thymol:*

Thymol, a common antiseptic, was used in one test, the results of which are presented in Table LXXIX. The gains in sugars and synthetic efficiencies are recorded

TABLE LXXIX

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT THYMOL (3 GM/1) FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	71.95±0.062	1.094±0.018	3.548±0.006	4.830±0.011
Glucose	72.13±0.033	1.655±0.011	5.554±0.000	7.502±0.010
Glucose + thymol	71.89±0.071	2.188±0.018	4.788±0.019	7.229±0.039

in Table LXXX, and the percentages of fructose and glucose in Table LXXXI. Table LXXX shows that thymol decreased the synthetic efficiency from 74.51 to 51.68. Table LXXXI shows that thymol increased the gain in glucose and decreased the gain in fructose, indicating that thymol interfered with the conversion of glucose to fructose.

TABLE LXXX

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT THYMOL (3 GM/1) FOR 24 HOURS, CALCULATED FROM TABLE LXXIX

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	2.692	2.006	74.51
Glucose + thymol	2.399	1.240	51.68

TABLE LXXXI

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT THYMOL (3 GM/1) FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.447±0.023		0.647±0.027	
Glucose	1.217±0.008	0.770	0.438±0.004	-0.209
Glucose + thymol	0.766±0.044	0.319	1.422±0.026	0.775

25. Sodium chlorate and sodium pentachlorophenate:

The effect of these chemicals upon synthesis was studied because they are commonly used as a weed killer. Sodium chlorate is readily absorbed by leaves or roots and is translocated in the xylem, killing the tissues. The exact mechanism of killing is not known. Crafts (13) stated that its effect may be due to its high oxidizing potential, to the presence of pentavalent chlorine, or to complete oxidation of respiratory chromogens. Hance (28) reported that chlorate liberates free or nascent oxygen, which supposedly destroys weed tissues. Gay (26) studied the effects of the H.S.P.A. Activator (sodium pentachlorophenate), and found that it promotes faster and deeper penetration of toxic chemicals into plants; it competes with the plant for carbon dioxide and oxygen, it decreases transpiration, and it destroys the enzyme diastase.

For the synthesis test, 0.2 per cent pentachlorophenate and 0.22 per cent chlorate were used. These weak concentrations were chosen so as not to kill the blades but to produce partially toxic conditions. The blades given pentachlorophenate were streaked with red along the veins and midrib for the lower third of the blade, while the blades given chlorate exhibited no discoloration. The results are recorded in Table LXXXII. The gains in sugars and the synthetic efficiencies are presented in Table LXXXIII, and the percentages of fructose and glucose in Table LXXXIV.

TABLE LXXXII

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT SODIUM PENTACHLOROPHENATE (0.2%) AND SODIUM CHLORATE (0.22%) FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	67.11±0.024	0.500±0.003	2.333±0.005	2.956±0.002
Glucose	64.57±0.029	1.531±0.003	5.439±0.008	7.256±0.011
Glucose + Na pentachloro-phenate	65.79±0.024	2.070±0.019	3.991±0.019	6.271±0.039
Glucose + Na chlorate	64.31±0.033	1.467±0.019	4.558±0.032	6.264±0.014

TABLE LXXXIII

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT SODIUM PENTACHLOROPHENATE (0.2%) AND SODIUM CHLORATE (0.22%) FOR 24 HOURS, CALCULATED FROM TABLE LXXXII

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	4.300	3.106	72.23
Glucose + Na pentachlorophenate ...	3.315	1.658	50.01
Glucose + Na chlorate	3.308	2.225	67.26

TABLE LXXXIV

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES SUPPLIED WITH GLUCOSE WITH AND WITHOUT SODIUM PENTACHLOROPHENATE (0.2%) AND SODIUM CHLORATE (0.22%) FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	0.568±0.009		0.000±0.000	
Glucose	0.848±0.005	0.280	0.683±0.002	0.683
Glucose + Na pentachlorophenate ..	0.681±0.003	0.113	1.388±0.016	1.388
Glucose + Na chlorate	0.634±0.014	0.066	0.832±0.005	0.832

Both chemicals decreased the absorption of glucose as shown by the gains in total sugars. The pentachlorophenate decreased the synthetic efficiency and increased the gain in glucose more than the chlorate did. These findings indicate that both chemicals interfered with the conversion of glucose to fructose and with the synthesis of sucrose, and that the effect of pentachlorophenate was worse than that of the chlorate.

SUMMARY

The effects of the following inhibitory agents upon the interconversion of glucose and fructose and the formation of sucrose in detached blades of the sugar cane plant are reported herein: cyanide, pyrophosphate, azide, 8-hydroxyquinoline, iodoacetic acid, arsenite, selenite, fluoride, malonate, acenaphthene, chloroform, dinitrophenol, ethyl alcohol, histidine, phloridzin, quinine, urethane, brilliant alizarine blue, rosinduline GG, iodine, silver nitrate, copper sulphate, sodium diethyldithiocarbamate, potassium ferricyanide, thymol, sodium pentachlorophenate, and sodium chlorate.

Sodium cyanide (0.049 gms/liter) did not inhibit either interconversion or synthesis.

Sodium pyrophosphate (8 gm/liter) did not inhibit either interconversion or synthesis.

Sodium azide (0.05 gm/liter) did not inhibit either interconversion or synthesis, but did decrease the synthetic efficiency from 84 to 78.

8-hydroxyquinoline did not inhibit either interconversion or synthesis.

Since some of the more important inhibitors of iron-catalyzed reactions and copper-catalyzed reactions did not inhibit synthesis, it would seem that the oxidases, peroxidases, catalase, and other enzymes containing iron or copper are not involved in the synthesis of sucrose.

Iodoacetate (0.0001 M) did not inhibit either interconversion or synthesis, whereas a strong concentration (0.01 M) completely inhibited both interconversion and synthesis. This would indicate that the processes of fermentation beginning with triose phosphate dehydrogenase are not involved in the synthesis of sucrose. Some process which is inhibited by 0.01 M iodoacetate is essential for synthesis. This process may be the phosphorylation of glucose by hexokinase.

Sodium arsenite (100 p.p.m. As) completely inhibited both interconversion and synthesis.

Sodium selenite (100 p.p.m. Se) completely inhibited both interconversion and synthesis.

Detached blades supplied with water in the light accumulated glucose but not fructose, when given either arsenite or selenite. Since blades known to be supplied with glucose accumulate glucose, and blades known to be supplied with fructose accumulate fructose, in the presence of arsenite or selenite, the results obtained with blades in water in the light in the presence of these poisons, constitute strong evidence that the first sugar formed in photosynthesis is glucose alone.

Sodium fluoride (47 p.p.m.–380 p.p.m. F) depressed both interconversion and synthesis. The effect of fluoride was not as bad as that of iodoacetate, arsenite, or selenite, since even 380 p.p.m. F ($= 0.02$ M) did not inhibit synthesis completely.

Sodium malonate (0.5%) did not inhibit either interconversion or synthesis, but did decrease the synthetic efficiency from 68 to 58.

Acenaphthene (0.1%) appeared to aid both the conversion of glucose to fructose and the formation of sucrose.

Chloroform decreased both the conversion of glucose to fructose and the synthesis of sucrose.

Dinitrophenol (50–100 p.p.m.) appeared to diminish synthesis a little.

Ethyl alcohol (2–5%) appeared to raise the synthetic efficiency a little.

Histidine appeared to increase synthesis a little, particularly in 6×10^{-4} M concentration.

Phloridzin (0.01 M) had no effect upon either interconversion or synthesis.

Urethane (1%) had no effect upon either interconversion or synthesis.

Brilliant alizarine blue (4.9×10^{-8} M) inhibited synthesis. More glucose than fructose accumulated whichever sugar was supplied, indicating that brilliant alizarine blue did not inhibit the conversion of fructose to glucose but may have decreased the conversion of glucose to fructose.

Rosinduline GG (1.87 gm/liter) cut the synthesis of sucrose in half. More glucose than fructose accumulated whichever sugar was supplied, which indicates that

the dye had no deleterious effect upon the conversion of fructose to glucose but may have decreased the conversion of glucose to fructose.

Since dyes which are known to inhibit the conversion of hexose monophosphate to fructose diphosphate, have been found to prevent the formation of sucrose, it is concluded either that the dyes exert a specific effect upon synthesis or that phosphorylation of glucose must proceed as far as the fructose diphosphate stage for the synthesis of sucrose to take place.

Iodine, silver nitrate, and copper sulphate ($M/50,000$) each increased the synthetic efficiency a little. Neither iodine nor copper sulphate affected the conversion of glucose to fructose significantly, but the effect of silver nitrate in depressing the conversion of glucose to fructose may have been significant.

Since iodine, silver nitrate, and copper sulphate, all in $M/50,000$ concentration, are known to inhibit the breakdown of fructose diphosphate by zymohexase, it would appear that inhibiting the action of zymohexase aids the synthesis of sucrose.

Because inhibiting the formation of fructose diphosphate inhibits synthesis, whereas inhibiting the breakdown of fructose diphosphate increases synthesis, fructose diphosphate may be a stepping stone necessary for the formation of sucrose from glucose.

Sodium diethyldithiocarbamate ($2 \times 10^{-5} M$) had little if any effect upon synthesis. Since this chemical is known to inhibit succinic dehydrogenase, this enzyme evidently plays no part in the formation of sucrose from glucose.

Potassium ferricyanide (3.292 gm/l) interfered with the conversion of glucose to fructose and with the synthesis of sucrose.

Thymol (3 gm/l) decreased both the transformation of glucose to fructose and the formation of sucrose.

Sodium chlorate (0.22%) and sodium pentachlorophenate (0.2%) both depressed the conversion of glucose to fructose and the synthesis of sucrose. The effect of sodium pentachlorophenate was worse than the effect of sodium chlorate.

The Primary Index, Its Meaning and Application to Crop Management With Special Reference to Sugar Cane*

By HARRY F. CLEMENTS AND T. KUBOTA†

The single prime motivating force in the production of crops is atmospheric energy, in the forms of heat and light. Soil factors as well as certain internal plant factors tend to preclude the realization of maximum yields in any given area. Hence it is desirable for a grower to be able to follow some index within the plant which integrates these factors in relation to the available energy. This index is the Primary Index and is the total sugar level of the elongating cane sheaths expressed as per cent of the dry matter. The basis for the selection of this tissue is reported in part one of this paper.

The significance of the fluctuations of the primary index is reported in part two. As the external factors, sunlight and temperature, increase, the primary index rises. As they decrease, the primary index falls. To the internal plant factors, moisture and growth, the primary index is inversely related. As these fall, the primary index rises. In these studies the factors of soil nutrients such as K and P were at such high levels that their influences on the primary index were not discernible. Nitrogen influences the primary index indirectly as it affects the moisture level.

In part three, the use of the primary index in managing field crops is shown for seven crops.

INTRODUCTION

In 1940 the senior author published data showing the quantitative relationship between yield of sugar cane and the available atmospheric energy. In those data there were evident no indications of soil nutrient nor soil moisture deficiencies. The enormous differences in yield observed between the plantings at Waipio and Kailua‡ were traceable to differences in the intensity of sunlight and its absorption at the two locales. "Since differences in atmospheric conditions are so marked, it seems clear that the fertilizer requirement of a crop will differ from area to area and from year to year. Therefore, in order to best integrate the growth of cane with the unpredictable weather, it is necessary to follow some index within the plant which will describe its reaction to the various elements of its surroundings." (3, p. 150.)

Since the publication of that paper, many more data have been compiled, which now may be used to elaborate upon and substantiate the general concept as stated above. In approaching the production of crops from this biodynamic view, the grower is confronted with the realization that the single, prime motivating force in

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‡ Waipio and Kailua are located on the island of Oahu and are about 15 air miles apart. Waipio is on the leeward side and is in a high-sunlight, low-rainfall area. Kailua is on the windward side and is in a low sunlight, moderate rainfall area.

the growth of plants is the energy available to them from light and heat. Thus the theoretical maximum yield of a given crop in a given area is determined by the energy available to the crop. Once that yield for a given variety is reached, the addition of fertilizers to the soil to increase further the intensity of nutrition is pointless and wasteful.

To achieve these maximum yields requires the complete absence of deterring factors. Such factors may be both internal and external. The internal factors include the restrictions placed upon the whole plant by its conductive systems, leaf structure, root expanse, protoplasmic sensitivity to high energy levels, the variously induced idiosyncrasies of the organism, etc. The external factors which are deterrents to maximum efficiency are moisture insufficient to permit the plant the maintenance of a happy balance with the energy it is receiving, insufficient soil nutrients interfering with the normal integration of the physiological processes operating within it, insufficient soil oxygen and heat interfering with the respiration of the absorbing capacity of the roots thereby interfering with their work of growth, water and nutrient absorption, the presence in the soil of materials toxic to the particular plant, biotic factors such as disease organisms, weeds, rodents, insects, and certainly not the least, man. Whatever the deterring factor may be, the plant reacts to it and as a result its efficiency as a biodynamic organism is interfered with. In many cases when circumstances are less than favorable, the plant undergoes internal reactions which tend toward preserving its life but at the expense of a greatly reduced efficiency (2).

With such myriads of factors, all of them deterrents to the achievement of maximum efficiency, the plight of the grower might be regarded as hopeless were it not for the fact that the plant itself is a sensitive organism and as such reflects its own well-being. Furthermore we may assume a certain spontaneousness on the part of the plant. If external and internal factors are both favorable, we can assume that the plant will grow. Within each species of plants it should be possible to locate a process or index which is sensitive to the balance existing between the external energy and the internal reaction to that energy. As the intensity of the atmospheric energy varies, the plant reacts to it and the new state of balance arrived at should be reflected by the primary index. When such a primary index indicates undesirable trends, the specific secondary indices reveal the cause. In this way the plant itself is informing the grower of its hazards and, when such deterring factors are possible of correction, the grower may come close to realizing maximum returns from the energy available.

Perhaps even more important is the fact that in low-energy areas, where high yields are impossible, relatively high efficiency, however, is possible. The attention of a grower in a low-energy area should not be directed toward the high yields achieved in high-energy areas, but rather toward the efficiency achieved by his own crops under his particular weather and energy conditions. When such a grower sees how much fertilizer and water are used in high-energy areas, he is inclined to use the same quantities. Such practices are not only futile and wasteful, but are frequently injurious to quality, as will be shown presently.

It is fortunate for this type of work that apparently there is no energy area in the Territory which exceeds the growth and assimilation capacity of sugar cane; as a matter of fact, many areas are considerably below such maxima.

Early in these studies it became apparent that the temporary storage of sugars

in such organs as the leaf sheaths was correlated with the balance between photosynthetic production on the one hand and utilization or growth on the other, as related to the environmental condition. Thus as the total sugar level of the sheaths was maintained at about ten per cent (dry weight), there appeared to be a balance between these two contrasting processes. As the sugars dropped below that general level, it was apparent that the growth rate was relatively greater than the rate of photosynthetic production. Such a condition usually obtains during periods of high temperatures, both night and day, especially when associated with conditions of excessive tissue hydration and cloudiness or low light intensity. Obviously under such conditions the sugar content of the cane itself does not keep pace with the addition of new cane. Further, if the growth rate is of such magnitude that it greatly exceeds photosynthetic production, even the permanent reserve which was already laid down is drawn upon for growth. (It should not be presumed that in the life of the plant such conditions are not at times desirable.) It must be appreciated that any deficiency which will directly interfere with photosynthesis will also result in a reduction of the sugar level of the sheaths. Fortunately, however, such cases seem very rare. In culture solution, where iron or magnesium deficiency are induced, resulting in marked chlorosis, even though the growth rate is very low or perhaps even at zero, the sugar level drops very much below the ten per cent level. In the field there appears to be a condition which is sometimes observed in young crops, seemingly brought on either by a combination of inadequate irrigation and inadequately available nitrogen, or by waterlogging, especially on the heavy adobe soils. In a very young crop this combination expresses itself in a very marked yellow-browning of the leaves. As might be expected this chlorosis will impair the photosynthetic efficiency, hence the total sugars of the leaves will drop, even though growth may be at a standstill. However, this condition is so easily detected either superficially or by the moisture and nitrogen indices that it should cause no difficulties.

As the sugar level of the sheaths rises, it is indicative in general of the reverse condition. When conditions are such that the photosynthetic production exceeds utilization of the product, there is an accumulation of sugar in the sheaths. Circumstances bringing about this condition are low temperatures associated with bright weather, low tissue hydration induced either by less than adequate rainfall or irrigation or by very low nitrogen levels within the plant.

OBJECTIVES

The objectives of this paper are threefold:

- (1) The selection of the most reliable tissue within which the process of temporary sugar storage correlates with the factors of sunlight, temperature, moisture, growth, and such other factors as may affect the biodynamic balance between the plant and its total environment. Thus the level of sugar in that tissue will be the primary index.
- (2) The meaning of the variations observed in the primary index.
- (3) The application of the primary index in crop management.

EXPERIMENTAL

The general experimental procedure has already been described (4, 5). The data on which this paper is based are a part of the general project on which earlier papers were published.

Carbohydrates were fractionated into reducing sugars, sucrose and hydrolyzable polysaccharides. For the reducing sugars and sucrose, two-gram samples (dry and finely ground) were extracted under reflux with 80 per cent ethyl alcohol for three hours in a water bath. The alcoholic extract is filtered off the residue, and evaporated to near dryness under reduced pressure. The resulting residue is taken up with water, made to volume, cleared with lead acetate and delead with disodium phosphate. Aliquots of the cleared solution are taken for inversion with invertase scales. Aliquots of the first solution as well as of the inverted solution are taken for the determination of reducing sugars and total sugars following the method of Quisumbing and Thomas (1, pp. 138-139). Sucrose is determined by difference. In this paper, only the total sugar data are used.

DATA AND DISCUSSION

Before proceeding toward the realization of the objectives of the paper, it is necessary to list the more important factors which influence the primary index. Since the function of the primary index is to integrate the factors which will describe the balance within the plant in relation to its environment, it is clear that some of these must be external and others internal. The most important external factors are, of course, sunlight and air temperature. Moisture may also be included as an external factor, but here it is treated as an internal factor. As has already been reported, the moisture content of the elongating cane sheaths is a very reliable index to the moisture status of the plant (4), and is itself the result of an integration by the plant and includes many factors such as available moisture, relative humidity, wind, vitality of the roots, etc. Since the primary index supposedly reflects the balance between photosynthetic production and utilization by the plant, then obviously the growth rate must be included. Finally the three most commonly deficient soil elements, nitrogen, potassium, and phosphorus are included. These factors appear to be sufficiently inclusive so that correlations with the primary index are possible. Other factors undoubtedly come to mind. The concentration of chlorophyll is one such. Chlorophyll determinations were not made, but superficially, at least, the chlorophyll concentration is correlated with the total nitrogen and moisture.

Measurements of each of the seven factors mentioned above are obtained as follows:

Sunlight: The intensity and duration of sunlight are measured by obtaining the daily difference between the curves obtained from black-bulb and white-bulb distance soil thermographs. The black bulb is exposed directly to sunlight and the white bulb is placed within a regulation weather kiosk. The difference between the two curves is measured by means of a planimeter and converted to sunlight-degrees by dividing the area so measured by the area of 1° F. for twelve hours on the thermograph chart. Admittedly this is a crude measure of light. However, it has shown itself to be useful. The nature of the plant reaction to these values is in the same direction as for gram-calories per cm.² per second as determined by a pyrliometer, although somewhat weaker. Thus the relationships to be reported later may be regarded as conservative.

Temperature: The daily temperature values are compiled on the basis of weighted average values for a full 24-hour day and are read from the chart of the white-bulb thermograph. The area below the temperature curve is obtained for each 24-hour

period and divided by the area of 1° F. for 24 hours on the chart. In this way a fully weighted average for the entire day is obtained. Both the black-bulb and white-bulb thermographs are checked once each five weeks against a standard mercury thermometer. To accomplish this most reliably, it is necessary to lower both bulbs into a container filled with water. When equilibrium has been reached, the temperature of the water as reflected by the mercury thermometer is used as the standard, and corrections of the thermographs are made when necessary.

Moisture: The moisture content of the elongating cane sheaths (the moisture index) expressed as the per cent of the green weight was used as the measure of the water relations of the plant. Expressing the moisture content on the dry weight of the tissue was found to be wholly undesirable since very large distortions were introduced.

Growth: The growth values for the crops are average elongations expressed as cm. per day. The measurements are made on twenty pilot plants in each plot, the same pilots being maintained throughout the crop cycle. Occasionally a pilot plant is destroyed by rats or accidental breakage and is abandoned. When this occurs early in the crop, a new plant is selected. The natural brown mark on the dorsal side of the topmost sheath bearing a visible dewlap is used as the measuring point.

Nitrogen: The nitrogen index of the plant was used as a measure of the intensity of the nitrogen nutrition. As already reported, this index is the total organic nitrogen content of the elongating cane blades expressed as per cent of the dry matter.

Potassium: The potassium index of the plant is the potassium content of the elongating cane sheaths expressed on the basis of the sugar-free dry weight. The data on which this statement is based will be published shortly.

Phosphorus: The phosphorus index of the plant is the phosphorus content of the elongating cane sheaths expressed on the basis of the sugar-free dry weight. The data on which this statement is based will be published shortly.

Since we are dealing with a rather large complex, it must be apparent at the outset that the task undertaken is no small one. Certainly every one of the plant factors listed above is somewhat dependent on all the other factors, both external and internal. Hence we are not dealing with simple or independent relationships. In one plot the whole course of the crop may be determined by a single factor. In another the course may be determined by another single factor or by a combination of two factors, or three, or four, etc. To proceed by trial and error to discover the situation in each plot leads to tremendous numbers of calculations and, needless to say, defeats. It occurred to us that were we to combine all seven factors at one time and use the method of multiple regression (7), we could determine not only the individual correlations and standard partial regressions of each, but the final multiple regression coefficient (R). From these statistics the relative importance of each factor in each plot might be ascertained. By using these seven factors against the total sugar levels of each of the possible index tissues, we should by the level of R and by the apparent reasonableness of standard partial regressions select the most reliable index tissue and also determine the significance of the primary index as a tool for the guidance of the grower.

The Selection of the Primary Index Tissue:

The primary index tissue has essentially the same requirements as any other index tissue (5), but in addition it should be sensitive to the factors affecting the balance between the physiological processes of the plant as related to the environment in which the plant is growing. The possible tissues meeting all these requirements are the green-leaf sheaths, green-leaf blades, the elongating cane sheaths and the elongating cane blades.

In the earlier phases of this study the first five factors listed above were used. Because of the tremendous number of calculations involved, it was considered most advantageous to select the index tissue at this stage of the study and thus reduce the amount of mathematical work. The R values resulting from the multiple regression were calculated for each of the four possible tissues for each of the sixteen plots and are reported in Table I. Following these calculations, the most important factors in each crop were selected. These were selected as the ones making the greatest contributions to the R values and are reported in Table II.

TABLE I
MULTIPLE CORRELATION COEFFICIENTS¹
(5 Factors))

	Elongating cane sheaths	Green-leaf cane sheaths	Elongating cane blades	Green-leaf cane blades
Waipio:				
Plot A†	.8696†	.8667†	.6722	.8340*
B	.8835†	.9175†	.8237*	.9102†
C	.9045†	.7854	.7590	.8403*
D	.7519*	.8382†	.7484*	.7685*
RA	.8869†	.8692†	.8653†	.8233†
RB	.7289*	.8555†	.6973	.6469
RC	.6637	.6609	.6280	.7181
RD	.4921	.8056*	.6806	.6432
Kailua:				
Plot A	.7424	.6866	.6231	.4489
B	.9002†	.9229†	.7302	.8902†
C	.8009*	.8487†	.7773*	.7500*
D	.9189†	.8891†	.8318†	.9181†
RA	.7124	.8286†	.7336*	.8906†
RB	.7721†	.8269†	.8599†	.7809†
RC	.8027†	.7495*	.6580	.6394
RD	.5709	.5944	.7648*	.5779

¹ No mark indicates no statistical significance.

* Indicates statistical significance between the 5 and 1 per cent levels.

† Indicates statistical significance beyond the 1 per cent level.

‡ Plots A, B, C and D at each place are plant crops and differ from each other in the time of planting. Plot A was planted July 28, 1938. The others followed at intervals of three months. Plots RA, RB, etc., are the ratoon crops following the corresponding plant crops.

TABLE II
MULTIPLE CORRELATION COEFFICIENTS
(Most Pertinent Factors)

	Elongating cane sheaths	Green-leaf cane sheaths	Elongating cane blades	Green-leaf cane blades
Waipio:				
Plot A†	.8463†	.8588†	.6704	.7453*
B	.8814†	.9170†	.8166†	.9092†
C	.9042†	.7846†	.7443*	.8347†
D	.6863*	.7900†	.6716*	.5827
RA	.8563†	.8151†	.8501†	.7588†
RB	.7259†	.8390†	.7097*	.6712*
RC	.6626†	.6497*	.6038*	.6424*
RD	.4855	.7072*	.6657*	.6441*
Kailua:				
Plot A	.7382*	.6526	.5949	.4730
B	.8993†	.9083†	.7238*	.8747†
C	.7939†	.8098†	.7477†	.7108*
D	.9142†	.8480†	.8108†	.8910†
RA	.6786†	.8258†	.7249†	.8458†
RB	.7700†	.8215†	.8587†	.7784†
RC	.7678†	.7105*	.6569*	.6257
RD	.5426	.5929	.7542†	.5081

(* , †, ‡—See explanation in Table I.)

An examination of the significance of the multiple correlation coefficients in Tables I and II shows the rather marked superiority of the sheath tissues over the corresponding blade tissues and we need not concern ourselves further with the latter.

The choice between the elongating cane sheaths and the green-leaf cane tissues is not an easy one to make since both seem to be exceptionally good for the purpose of the primary index. Table I shows a slight superiority for the green-leaf cane sheaths over the other where all five factors are involved in the calculations. Table II, showing the most pertinent factors, shows the two tissues to be of about equal value. These values are, of course, quantitative, but when the qualitative aspect of each multiple correlation is examined, it is apparent at once that the green-leaf cane sheaths are considerably more sensitive to the moisture, often to the near exclusion of the other factors.

Such a situation is not desirable in an index which should tend toward equating all the factors in the plant's environment. The leaf blades are similarly overly sensitive to the moisture level. Such sensitivity is indeed curious, for the moisture level used in this study is that of the elongating cane sheaths. It will be remembered that the moisture levels of the green-leaf cane sheaths and the green-leaf cane blades were markedly inferior to those of the elongating cane sheaths as the moisture index (4). Yet the total sugars of the old sheaths and blades are more nearly related to the moisture index than is the tissue on which the moisture index is based. Probably the explanation is to be sought in the commonly observed fact that older leaves of plants are marginal in the moisture sphere of the plant, and hence it is there that processes are most affected by the moisture level of the plant. At any rate it seems reasonable that such tissues are less desirable as a primary index tissue than are tissues which, although as reliable, give less weight to a single factor. It is clear, of

course, that such factors as sunlight, growth, nitrogen and temperature are all pertinent to the energy equilibrium which is sought in the total sugar level. For these reasons the total sugar level of the elongating cane sheaths remains, at least for the present, as that best suited to integrate the factors of the environment in relation to the plant, and is therefore selected as the primary index.

The Meaning of the Primary Index:

Before using the primary index in managing a crop, it is necessary to be aware of the meaning of the variations within it. At the present stage it is not possible to give a complete picture of its equating capacities since not all the possible environmental circumstances have been encountered during the past four years in the sixteen crops which were grown. However, the influences of sunlight, temperature, growth and moisture seem clearly established. An important start has been made toward an understanding of the influences of the nitrogen, phosphorus and potassium levels upon the behavior of the plant. The problems of the influences of low calcium and magnesium as well as high calcium, magnesium and sodium are only now being undertaken.

In this section it is necessary to rely almost wholly on the statistical method of multiple regression (7). Using this method three statistics are derived, one, the simple correlation between each of the seven factors and the total sugars of the elongating cane sheaths, and two, the standard partial regression between each of the seven factors and the total sugars of the elongating cane sheaths. (The latter may be defined as the measure of regression between one factor and the total sugar when the variations of the other six factors [in this case] are neutralized.) Third, from these values, the multiple correlation coefficients (R) reported in Tables III and IV are obtained from the following formula:

$$R^2 = (r_{y1}) (\beta_{y0.123456}) + \dots + (r_{y6}) (\beta_{y6.012345})$$

Subscript 0 refers to the phosphorus index of the plant and is the P content of the elongating cane sheaths expressed as per cent of sugar-free dry weight.

Subscript 1 refers to the potassium index of the plant and is the K content of the elongating cane sheaths expressed as per cent of the sugar-free dry weight.

Subscript 2 refers to the sunlight record expressed as sunlight-degrees (see text for complete description).

Subscript 3 refers to the growth rate of the crop expressed as centimeters per day.

Subscript 4 refers to the nitrogen index of the plant and is the total nitrogen content of the elongating cane blades expressed as per cent of the dry weight.

Subscript 5 refers to the daily air temperature expressed as weighted averages per day in Fahrenheit degrees.

Subscript 6 refers to the moisture index and is the moisture content of the elongating cane sheaths expressed as per cent of the green weight.

y, in this case, of course, is the total sugar content of the elongating cane sheaths expressed as per cent of the sugar-free dry weight.

r is the simple correlation coefficient. β is the symbol of the standard partial regression coefficient and R the symbol of the multiple correlation coefficient.

Thus, r_{y0} , r_{y1} , r_{y2} , etc., are the simple correlations between the total sugar con-

tent and the phosphorus index, the potassium index, the light intensity, respectively, etc.

$\beta_{y0.123456}$ is the standard partial regression between the total sugar content of the sheaths and the phosphorus index when all other six factors are held constant. $\beta_{y1.023456}$ is the standard partial regression between the total sugar content and the potassium index when the other six factors are constant, etc.

R^2 is the sum of the products of each pair of correlations and partial regressions. R , the multiple correlation coefficient is obtained from R^2 .

In Table III the statistical analysis of the seven factors against the primary index is recorded for each of the sixteen plots. Further, the data for the plant crops (A, B, C, D) are combined for each place and similarly treated. Then the ratoon crops (RA, RB, RC and RD) following the plant crops are combined and analyzed. This is done in an effort to determine the outstanding factors influencing crop growth in the two regions. Unfortunately because of rat damage and blossoming, growth data for the ratoon crops at Kailua were not reliable and are not included.

An examination of the data in Table III reveals that some of the values (correlations and partial regressions) are very low. Some of these pairs have opposite signs (+ and -). Some, on the other hand, are high with the same signs. Some pairs are positive, others negative. Clearly some factors contribute much more to the value of R than others. The significance of R is determined by the analysis of variance. As a result, the larger the number of factors used, the greater is the value of R required for significance. This fact together with the obvious reason that we are chiefly concerned here with an understanding of the primary index requires that in each plot the factors of importance be separated from the others. Thus the most pertinent factors are selected for each plot, the partial regressions and multiple correlations recalculated. These are reported in Table IV. The actual values for R are always somewhat smaller than the corresponding values in Table III, but because of the smaller number of factors, the significance is relatively higher.

Before discussing each factor as it is related to the primary index, it may prove helpful to make a casual, over-all survey of Table IV. The columns headed phosphorus, potassium, etc., with the greatest number of blanks indicate least importance relative to the primary index—those with least blank spaces are the most important in being associated with the variations in the primary index. It is apparent that the most important are sunlight, growth, and water. The least important are phosphorus, potassium and nitrogen. It must be cautioned that this will probably be the case only where these elements are sufficiently abundant so as not to be deterring factors. To continue the general summary of Table IV, at Kailua, a cloudy, non-irrigated area, the data show that three factors alone account for most of the variation of the primary index—water being the most general, with sunlight and growth the other two. In no case are the factors of temperature, phosphorus and potassium associated with variations of the primary index and in only one case does nitrogen enter the picture. In other words whatever influences these factors may have played, their final effect on the sugar level of the sheaths was completely masked by water, sunlight and growth. To translate this discussion into field terms, during the periods when the primary index was high, the crop was suffering mostly from drought. In the later summer and fall, after rains came, the index was low because of excessive moisture

TABLE III
CORRELATION, PARTIAL REGRESSION AND MULTIPLE CORRELATION COEFFICIENTS OF SIXTEEN CROPS

	Phosphorus— r_{10}	Potassium— r_{11}	Sunlight— r_{12}	Growth— r_{13}	Nitrogen— r_{14}	Temperature— r_{15}	Water— r_{16}
	$\beta_{10.123456}$	$\beta_{11.023456}$	$\beta_{12.013456}$	$\beta_{13.012456}$	$\beta_{14.012356}$	$\beta_{15.012346}$	$\beta_{16.012345}$
Waipio:							
Plot A†	-.6451	-.3513	-.0872	+.4854	+.4343	+.4220	-.4459
B	-.0696	+.0794	-.3162	+.1361	+.3354	+.1684	-.6453
C	+.3206	+.3920	-.2490	-.1428	-.2042	+.0911	-.6878
D	+.2382	+.3372	+.0949	-.5594	-.0516	+.1871	-.4304
RA	-.2840	+.1727	-.5822	-.3793	+.4402	+.4548	-.3133
RB	-.4776	-.0032	-.5401	+.1669	+.4582	+.1563	-.2815
RC	-.3670	+.4283	-.6023	-.4610	+.0224	-.0531	-.4366
RD	-.2231	-.2028	-.1197	+.1953	-.0144	+.1612	-.3346
Plant Crops	-.0631	+.0204	-.1778	-.0428	+.1761	+.2416	-.5244
Ratoon Crops	-.3466	+.0408	-.4878	-.3846	+.2343	+.2243	-.3239
Kailua:							
Plot A	+.2321	+.3469	-.4032	+.1886	+.6531	+.4029	-.0017
B	-.1420	-.3384	-.2882	+.2366	+.5876	+.2603	-.6128
C	+.0769	+.3090	-.0824	+.2155	+.4629	+.2168	-.5103
D	-.2614	+.1864	-.1993	+.0576	+.5602	+.2943	-.3813
RA	-.4983	-.0531	-.2040	+.4427	+.3883	+.2333	-.4090
RB	-.3126	+.1435	-.5407	+.4153	+.5207	+.4790	-.4713
RC	-.1753	+.3817	-.3556	+.2981	+.4937	+.4475	-.1871
RD	-.0258	-.2105	-.1421	-.2069	+.5349	+.5008	+.0383
Plant Crops	-.0691	+.1792	-.1896	+.1453	+.5332	+.2337	-.4105
Ratoon Crops	-.2706	-.0843	-.3477	+.1239	+.4552	+.4719	-.2236

(*, †—See explanation in Table I.)

and growth and cloudy weather. The phosphorus and potassium levels were such that at no time were they associated with the variations of the primary index.

At Waipio where there is abundant atmospheric energy and where irrigation is practiced, the most important factor by far is growth, followed by temperature, sunlight, water, potassium, nitrogen and phosphorus. The nitrogen and phosphorus levels are associated with the variations of the primary index only in the plant crops. Potassium accounts for a fairly large part of the variations in the ratoon crops.

It may be profitable now to describe the influences of each factor upon the primary index somewhat in the order of importance.

Growth: In every case both the simple correlation and partial regression coefficients are negative. The reader, of course, would anticipate this relationship. As the growth rate speeds up, more and more carbohydrate is used up and hence the temporary reserves such as the total sugars of the sheaths (the primary index) will decline. Other things being equal when the growth rate declines, the reserves will rise. This relationship is one of cause and effect, as any grower well knows.

At Waipio in both the combined plant and the ratoon crops, growth is the single most important factor in accounting for the variability of the primary index. The combined plant crops at Kailua, however, show that growth was the least important of the three factors, the most important being water. These relationships signify that at Waipio, the crops, on the whole, were nearer maximum efficiency than were those at Kailua where water was an important deterring factor.

Water: In all cases the simple correlation and partial regression coefficients are negative. When the moisture level of the plant is high, the processes within the plant requiring carbohydrates may proceed until interfered with by other factors. Furthermore when the moisture level is high, the maximum levels of nutrients which the soil can provide are achieved. There is a very high positive correlation between water and nitrogen, potassium, and phosphorus, respectively. So strong are these relationships that one is forced to wonder at the reliability of results obtained in fertilizer trials when the water level of the crop is not controlled. Another aspect of these water-nitrogen relationships is seen when soil water is abundantly available—if the nitrogen levels of the plant and soil are near minimum, the moisture level of the plant remains low until the nitrogen level is raised. This fact is of considerable importance to growers in the high-rainfall areas who generally cannot depend on dry weather to mature their crop. Were these growers to reduce the nitrogen application to levels somewhat lower than needed by the crop, they could dry out their crops, at least throughout a large part of the year—even during wet weather! (See Kailua ratoon crop logs in next section.)

In Table IV the moisture levels of the Waipio crops (irrigated) were less frequently unfavorable than at Kailua. Only in Plots B and C and RB and RC does moisture account for some of the variations in the primary index. Undoubtedly this is so because the crops were thoroughly dried out in summer, prior to harvest. At Kailua, however, where irrigation was not available, water had a much more general effect on the primary index. Recurring droughts, especially during the plant crops, caused unseasonal rises in the primary index.

Thus because of the important role played by water in the life of the plant, both as to its own internal processes as well as its ability to obtain soil nutrients, it is apparent that whenever the primary index is high, the first possible cause to examine

TABLE IV

MOST PERTINENT CORRELATION, PARTIAL REGRESSION AND MULTIPLE CORRELATION COEFFICIENTS FOR SIXTEEN CROPS

	Phosphorus— r_{y0}	β_{y0}, δ	Potassium— r_{y1}	β_{y1}, δ	Sunlight— r_{y2}	β_{y2}, δ	Growth— r_{y3}	β_{y3}, δ	Nitrogen— r_{y4}	β_{y4}, δ	Temperature— r_{y5}	β_{y5}, δ	Water— r_{y6}	β_{y6}, δ	R
Waipio:															
Plot A†	-.6451	-.4092	+.4343	+.1070	-.4459	-.5154	-.7122	-.2287	+.2233	+.51019034†
B	+.3354	+.2073	-.6453	-.5304	+.6231	+.43468903†
C	-.6318	-.3520	-.3041	-.30419354†
D	-.4304	-.8949	-.47758187†
RA9064†
RB7314†
RC7062†
RD4466
Plant Crops8398†
Ratoon Crops6306†
Kailua:															
Plot A7271*
B5899
C7908†
D9126†
RA7740†
RB7584†
RC5851*
RD5415
Plant Crops8276†
Ratoon Crops6197†

§ The remainder of the symbol varies with each plot.

(* , †, ‡—See explanation in Table I.)

is the moisture level. If the moisture level is low, soil moisture is the most likely limiting factor. If the soil moisture is adequate, and still the moisture level in the plant is low, then the plant is suffering from a nutrient deficiency (most probably nitrogen) or from inadequate soil aeration and low soil temperature. The last two factors are common causes in adobe soils following prolonged periods of heavy rainfall. Frequently the plants lose their green color and become brownish-yellow. Under such conditions the primary index will become very low and might cause confusion were not the cause so obvious. Another cause of low moisture levels in the plant when soil conditions are favorable are high wind velocities associated with low relative humidities usually occurring during the spring months.

Sunlight: Table IV shows that without exception the correlation and partial regression coefficients between sunlight and the primary index are positive. That is, over the range of light intensities encountered by these crops, the greater the light intensity, the higher is the sugar level, other things being equal. It is common knowledge that beginning in February and sometimes extending well into July, juices are generally good. From July on, however, juices begin to deteriorate. In part this situation is explainable on the basis of sunlight and temperature and in part on the basis of moisture as affected by sunlight and temperature. In general the duration and intensity of sunlight increase from the first of the year until July, after which both decline until near the year's end. Associated with the increasing light intensity in the first half of the year but lagging behind it is the air temperature (6). Thus during this period the plant is building carbohydrates at a rapid rate, but because of the lower temperatures growth is restricted, hence these carbohydrates tend to accumulate. Further, because of the low temperatures, there is a tendency to underestimate the moisture needs of the plant, especially since during these cool, bright days the relative humidity of the air is low. All of these things combine to raise the primary index and also the quality of cane juices.

During the second half of the year, the situation changes strikingly even though gradually. As the intensity and duration of sunlight decrease, air temperatures continue to rise. Here radiant energy is lagging behind heat energy. Nights are warm, days are hot, and the relative humidity is higher in general than in spring. Coupled with this situation is the tendency toward more frequent irrigations resulting in high tissue hydration, rapid growth and, of course, poor juices. During this time of the year, good yields of good quality can only be effected through proper control of nutrient levels (especially nitrogen), and moisture.

The generally higher importance of sunlight at Kailua in relation to the primary index points to the obvious conclusion that the low intensities observed are the reasons for the generally low yields obtained. At Kailua when moisture is available, the primary index is low because the growth made, even though much less than at Waipio, is excessive in relation to the energy available.

Temperature: Within the range of temperatures recorded during these studies, the effect of temperature on the primary index is, with one exception, positive. This observation may be difficult to reconcile with the statement regarding temperature in the last paragraph. The positive correlations and partial regressions simply mean that within the temperature range observed, other things being equal, the higher the temperature the higher the total sugar level. However, the higher the temperature

the higher is the growth rate, and since the growth rate has a larger influence on the primary index, the influence of temperature is usually masked.

Nitrogen: The influence of nitrogen upon the primary index is somewhat more involved. In Table IV, at Waipio, nitrogen was an important factor in three cases, all plant crops. Here its effects are negative. But in these cases the nitrogen applied was excessive. When the nitrogen was applied as needed by the ratoons, it disappears as an important factor. At Kailua the nitrogen applied to the plant crops was also excessive but because of the dominant effect of moisture, nitrogen was of no significance in relation to the primary index. Kailua Plot RA, however, shows nitrogen to be significant because of its high positive partial regression. Thus although the correlation was very weak and negative, the partial regression was positive and very important. Its importance in this case is complementary to the effect of moisture. This influence will become clearer when the crop log is examined in the next section.

It perhaps may be helpful, however, to indicate some of the general influences of nitrogen observed in these studies. Reference to Table III will show that the simple correlations between nitrogen and the total sugar levels are for the most part negative. Where they are positive, they are usually weak. Six out of eight partial regressions for the plant crops, where the nitrogen applied was excessive, are negative. In the ratoon crops where the nitrogen applied was more nearly that needed, six of the eight partial regressions are positive, the other two negative. To be sure, most of these values are not significant and were it not for the fact that the nitrogen question is so often raised by growers, especially in those regions subject to periodic droughts followed by excessive rainfall, it would not be discussed here.

The probable cause-and-effect relationships here deal with the supply of energy available to the crop and secondly, the availability of moisture in relation to the energy available. In ordinary field terms these relationships may be expressed somewhat as follows: when there are available generous supplies of energy (both radiant and heat coupled with sufficient moisture, the effect of nitrogen is to force growth and, therefore, it tends to have an inverse effect upon the primary index. On the other hand when the supply of energy, particularly heat, is relatively low or when plants are experiencing deficiencies of moisture, the final effect of nitrogen is to be directly related to the primary index. Actually what may superficially appear to be a dual effect is really one effect. Common experience tells us that where nitrogen is limiting, the leaves are less green than where nitrogen is abundant. Other things being equal, abundant nitrogen thus implies greater vigor, higher metabolism, more chlorophyll, more photosynthesis. Hence under conditions of high temperature and moisture, increased nitrogen is correlated with increased growth and respiration. Under these conditions the stimulating effect of nitrogen on the rate of carbohydrate accumulation is not apparent. But a plant high in nitrogen growing under conditions which will restrain growth, more than it will restrain photosynthesis, will show a direct relation between nitrogen content and carbohydrate accumulation.

This effect has been noted by growers in the low-energy areas, and sometime disastrous results obtain. In cold, cloudy areas which have the additional hazard of drought, the thought has developed that by fertilizing a field heavily, greater sugar yields will result. Also, several growers have felt that applying nitrogen during a period of drought will result in increased yields. Table III shows positive evidence

supporting this contention, but as many of these growers know, the risks involved here are tremendous—greater than justify the practice. The effect of this treatment is essentially mild during the drought, but when the drought is broken, especially during periods of high temperature, there is only one result. The flush of moisture through the dry, well-aerated soil carries the nitrogen with it into the plant and once again the more ordinary effect of nitrogen comes into play—very rapid growth, greatly in excess of the radiant energy, and not only is there much absorption of water by the crop because of the excess nitrogen but in addition there is an actual drain upon the stored carbohydrate so painstakingly accumulated during the period of drought. The result of this may be a large tonnage (much of which is pure water) but a poor yield of sugar.

Potassium: The influence of potassium on the primary index is not discernible in these studies, primarily because the levels were generally high. Plot D at Waipio shows a weak positive correlation but a strong negative partial regression. The importance of this was to strengthen the partial regression of nitrogen. However, when the plant crops of Waipio were combined, no such relationship appeared. At no time at Kailua was there any indication that potassium may be a limiting factor. The Waipio ratoon crops show a negative relationship between potassium and the total sugars. There appears to be no correlation between the potassium levels and growth. It is difficult to determine whether the relationships between potassium and total sugars are cause or effect. Other studies dealing with the influences of potassium are under way, however. Since the relationship is negative, it would mean that a deficiency of potassium would show itself in a high primary index and a low potassium index.

Phosphorus: Phosphorus, as a factor affecting the primary index, is not important in these studies. At Kailua where the phosphorus levels were generally lower than at Waipio, it was not associated with the variations of the total sugars. At Waipio it was of no importance in any of the ratoon crops nor in the combined plant or ratoon crops. In Plot A at Waipio it forms an important part but with an inverse influence. Plots C and D at Waipio show it as a direct influence. Special phosphorus studies which are now underway, indicate that the effect of marked phosphorus deficiency is toward a reduction of the sugar level, but the levels of phosphorus in the plants showing this deficiency effect are far below anything observed in the field studies being reported upon.

In summarizing this part of the paper, it may be stated that the primary index reflects in its variations the balance between the external factors of sunlight and temperature and the internal factors of growth and water. As the intensity of the external factors increases, the primary index rises, and as the intensity of the internal factors, growth and water, increases, the primary index drops. Here, then, seems to be a simple guide which the grower may follow in managing his crop. The factors of soil nutrients *when maintained at adequate levels* are apparently of no importance in influencing the primary index. The levels of R are generally higher for the plant crop than for the ratoons. It should be remembered that the plant crops were grown according to plantation methods whereas the management of the ratoon crops was determined by the primary index. As a result there was a reduction in the variations of the primary index of nearly 50 per cent at Waipio and 30 per cent at Kailua.

The Application of the Primary Index to Field Crops:

In this portion of the paper a series of case records will be presented which were compiled as the crops grew. In the plant crops no attempt was made to guide them on the basis of the primary index. These crops were handled according to plantation practices and were used to establish the various indices. As the work of chemical analysis and growth measurements progressed, the apparent sensitivity of the total sugars of sheaths to factors of the environment showed itself. Thorough studies of it convinced us that it, indeed, was the most important index of all. This realization caused a revision in our estimate of the situation. Indices such as moisture, nitrogen, phosphorus, potassium, calcium, etc., became secondary. By this, it is not to be presumed that we consider them of little importance, but rather that the primary index reveals the status of the plant relative to its environment. When the primary index indicates an unfavorable position, the secondary indices are looked to for causes. When the plant crops were harvested and the ratoons begun, we undertook to grow these crops and manage them entirely on the basis of the various indices. Hence, seven field records will be presented and discussed according to Index records and the strategy used in each case. Four Waipio ratoon crops will be presented first, then two Kailua ratoons, and finally one Kailua plant crop.

Waipio Plot RA:

In Fig. I the crop log* for the ratoon crop, Plot RA, is reported. The sunlight is expressed as sunlight-degrees averaged as daily averages for the five-week intervals. Next is the temperature record expressed as the weighted 24-hour average. The nitrogen index is based on the total nitrogen content of the elongating cane blades (per cent dry matter). The curve below it is the moisture index which is the moisture content of the elongating cane sheaths expressed as per cent of the green weight. The arrows below the moisture curve are dates on which irrigation water was applied. For Kailua, rainfall is plotted below the moisture curve. Below this is the potassium index which is the K content of the elongating cane sheaths expressed as per cent of the sugar-free dry weight. The next curve is the phosphorus index which is the P content of the elongating cane sheath expressed as per cent of the sugar-free dry weight. Below this is the primary index, which is the total sugar content of the elongating cane sheaths expressed as per cent of the dry matter. In the lowest section of the figure are the growth rates of the crop reported as the average in centimeters per day for the thirty-five-day interval. At the bottom are the dates on which collections of samples were made. For several of the curves a line labelled "tentative normal" is drawn across the sheet. These lines are thought to be near the levels which are still adequate for maximum crop yields, although minor adjustments will be made as more experience is accumulated. The normal line for nitrogen is based upon the analysis of the whole leaf. The normal for moisture is based on the known growth behavior of the crop. The normal for potassium and phosphorus is based on analysis made of cane crops grown for many years at Waipio from plots which have received no potash or phosphate fertilization, but which have maintained their yields. The normal for the primary index is an approximation resulting from the sixteen crops grown in this study. All of these "normals" should be regarded as

* The late Hamilton P. Agee suggested the name "crop log" for such field records. The term may be defined as a record of the crop's progress from its beginning to harvest.

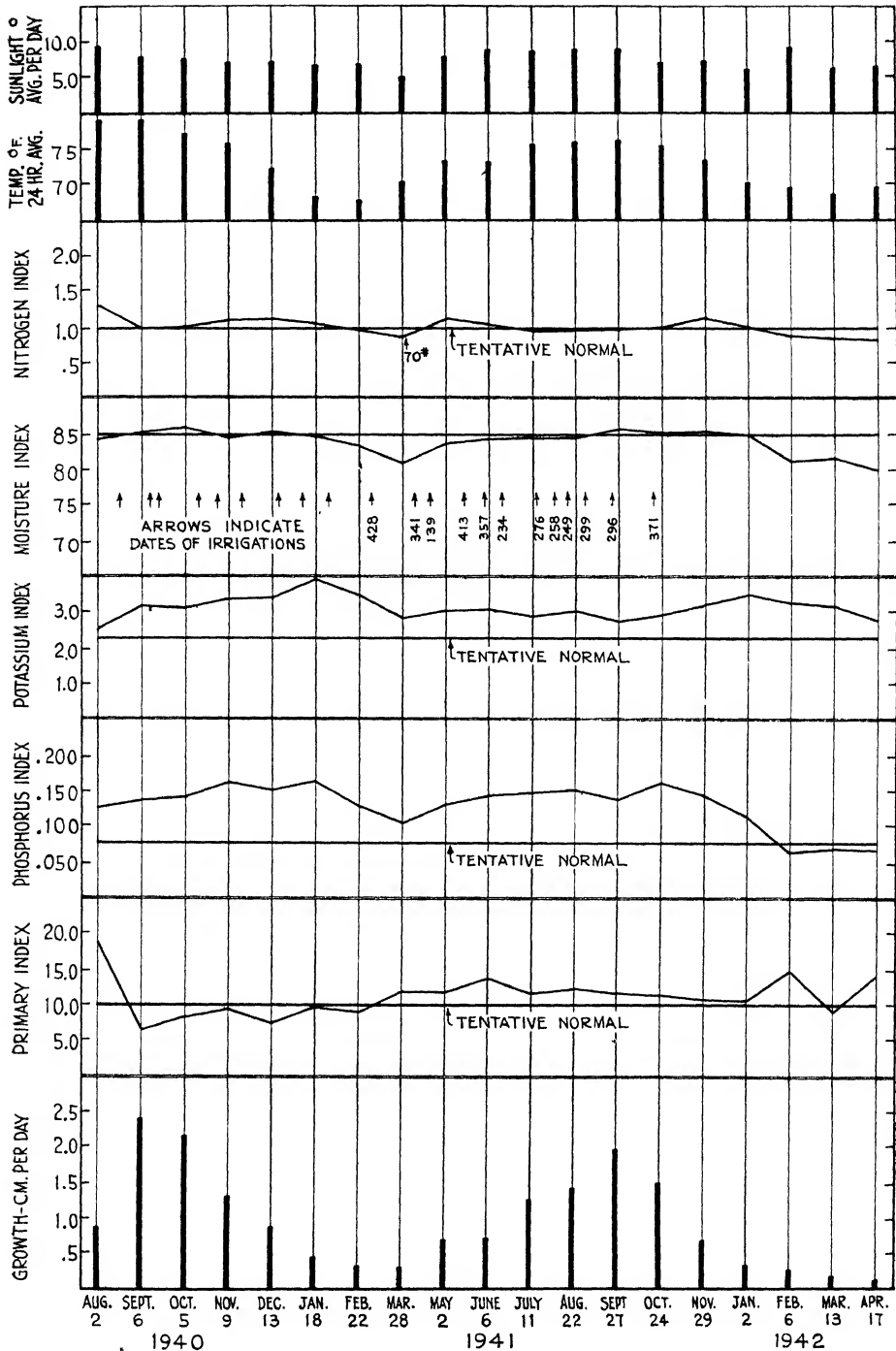


FIG. 1. CROP LOG FOR WAIPIO PLOT RA.

tentative, but at the same time they may be regarded as fairly close to the true normal.

The ratoon crop was begun June 1, 1940. It followed an excellent plant crop which had been well matured. The records for the plant crop showed a high phosphorus index and a moderate potassium index. Therefore, no phosphate was applied to the ratoon. Potash was applied at the rate of 200 pounds of K_2O per acre (muriate). This was done simply as a safety factor, since not enough information had been as yet obtained to omit the potash with confidence. Nitrogen was applied immediately at the rate of 100 pounds of nitrogen per acre as sulphate of ammonia. This seemed like a very heavy application, but later it will be seen that it was not unwise. Five irrigations were applied between June 1 and July 31. Further irrigations are indicated on the field record, as arrows under the moisture index. During the first part of the cycle, irrigations were made more or less by rule of thumb. Beginning in 1941 the Waipio substation placed its irrigation on a day-degree basis. After this the irrigation interval was regulated by the number of day-degrees experienced by the crop since the previous irrigation. The numbers under the irrigation arrows represent the accumulated day-degrees for each interval.

With these explanatory remarks, we may now proceed to follow the crop log as it was compiled.

August 2: The primary index was very high. However, this commonly occurs in the very young plants before they begin rapid growth and if moisture and nitrogen are adequate, it presents no cause for alarm.

September 6: The primary index dropped to a very low level and might be considered dangerous. At that time of the year, with intense heat, that can only mean excessive growth. Actually the growth rate was tremendous, averaging 2.4 cm. per day or nearly $2\frac{1}{2}$ feet per month. But at such an early stage of growth we need to take advantage of the growing conditions and force the crop as much as we can, for there will be several months of less favorable weather soon and during this period the crop can be hardened and prepared for lodging, and so we continue to push it. (Note: Reference to the nitrogen index on September 6, 1940, shows it to be dropping very rapidly. Were we basing our crop management solely on that curve, we would be inclined to apply more nitrogen. However, the primary index is very low indicating that nitrogen is not a deterring factor, hence no nitrogen is applied.)

October 5: The primary index is rising but still is well below the ten per cent line. Moisture is also rising, hence we can lengthen the irrigation interval. Nitrogen is rising. Since no nitrogen was applied, it means that the absorption of nitrogen is continuing at a high rate but since the growth rate is slowing, the nitrogen is at a higher level in the plant.

November 9: Growing conditions are worsening. The primary index is still rising but is approaching normal. Nitrogen continues to rise. Moisture is normal. No change in management is made.

December 13: Cloudy weather is upon us. The primary index drops. Moisture is normal. Nitrogen still rising. No change is made.

January 18: The crop now is in cold weather. Growth for the next two or three months is likely to be very slow. The crop is nicely erect but is becoming tall. We may now take advantage of the cold weather to harden the crop without losing any important growing time. Further, we want the crop in such a position that we can

again force it the coming summer. But if we do not harden it before it lodges, we may have it go down during a storm with resulting losses due to breaking and burying, as experiences in the plant crops had demonstrated. Thus we cut irrigation frequency and impose a drying period upon the crop.

February 22: Evidence that drying is occurring is obtained from the moisture and nitrogen indices. The primary index is still somewhat below normal. We continue the reduced irrigation applying water after 428 day-degrees.

March 28: By this time drying is very pronounced. The primary index has risen above normal. Moisture is low and nitrogen is low. The season is advancing into favorable weather for growth again. Here, then, we reach another period requiring a decision. We can apply more water, but it is also clear that nitrogen will become a limiting factor, hence we make the final application of nitrogen. The quantity to be added must be decided upon. The crop so far has received only the original application of 100 pounds. We know the sunlight which the crop has experienced to date. We look ahead and make a conservative estimate of the sunlight which the crop is likely to experience. To be doubly certain that the estimate is conservative, the last five months of the crop are not included in the estimate. We reason that if 100 pounds of nitrogen carried the crop through the energy available to date, then 70 pounds should carry us through the forecasted amount. In this case the answer was 70 pounds. The 70 pounds of nitrogen were applied with water on the day indicated by the arrow under the nitrogen curve.

May 2: The primary index has stopped rising. Moisture and nitrogen have risen sharply. No change in program.

June 6: The primary index has risen sharply! Nitrogen is normal. Moisture is somewhat below normal. The crop is going down very satisfactorily but the primary index is too high. We change the irrigation program to a 250-day-degree interval.

July 11: The primary index is better this time. No change in program.

August 22: The primary index is a little high, but not seriously for the second year. With the rising temperatures looming ahead, there is a danger that the second boom stage may cause a lowering of sugar. To be on the safe side, we add 50 day-degrees to the irrigation interval. This is done with caution because the crop is now at a critical point. If its growth during the next two months is excessive, the quality ratio will suffer. If the growth rate is checked severely during the immediate period, there is danger of blossoming. Hence, only a mild lengthening of the irrigation period is effected.

September 27: The primary index is starting downward. Danger of floral initiation is pretty well passed by now for 31-1389. The moisture index is above normal. Growth is heavy. The irrigation interval is lengthened to 375 day-degrees.

October 24: The primary index continues downward! The moisture index is still high. We have our tonnage by now. The harvesting schedule calls for the harvesting of this crop early in April. The falling primary index may become serious, hence something drastic needs be done. The last irrigation was applied on October 13. Harvest is six months away with very uncertain weather between now and then. We decide to stop irrigations at once. If, after a month or two, the moisture index begins to drop sharply, we can always apply another irrigation.

November 29: The primary index continues downward. The moisture index is high. The nitrogen index has risen sharply. No change in program.

January 2: Still the primary index declines. Moisture has dropped a little but is still normal. (Here after nearly three months without irrigations and only minor showers, the moisture index is normal—certainly a saving.) But now harvesting is only three months away.

February 6: At last the primary index has risen sharply, maturation is underway. Moisture has dropped sharply, nitrogen is also dropping.

March 13: The primary index is down again. Moisture has risen only slightly. Nitrogen is down. Certainly there is nothing in these curves to indicate the behavior of the primary index. When the weather records are consulted, the reasons are clear—low temperatures, cloudy weather and showers. The primary index need not frighten us, however, since with both moisture and nitrogen down, a little good weather is all that is needed.

April 17: The primary index is well up, moisture is very low and nitrogen is low. We could extend the crop for another month, but it is practically ready for harvest now. It is harvested and yields 110.4 tons of cane to the acre. The quality ratio is 7.3. The sugar yield is 15.1 tons per acre.*

Summary: During the first part of the crop cycle, the primary index was kept low by extremely heavy growth and high temperature. During the middle part, the primary index was in general high due to sunlight intensity and small growth. In the very last of the crop, moisture and sunlight and temperature were chiefly responsible for the primary index. These observations are verified by the correlations and partial regressions for the plot shown in Tables III and IV. In producing this crop, twenty-six rounds of irrigation, 170 pounds of nitrogen and 200 pounds of potash were used. The potash probably was unnecessary since the potassium index was considerably above the tentative normal. The phosphorus index was also very high. Had another ratoon crop followed the present crop, there would have been no need for phosphorus or potassium fertilization. To reassure those who might question this statement, it may be pointed out that the phosphorus curve was much higher than that of the preceding plant crop despite the fact that no phosphate fertilizer was applied to the ratoon. So far as potassium is concerned, the curve is also considerably above normal, although in this case 200 pounds of potash were applied. Now were another ratoon being started, since the potassium level is known to be high, the new crop might be started without potash application. After the crop is underway for a while, an application can still be made should the potassium index indicate the necessity. However, with the level as high as it was, the chances for a need developing in the next crop are very small.

* The yield data reported at the end of each crop log are regarded as rough estimates designed to give the reader an idea of the kind of crop produced.

The tonnage figures of Waipio crops are based on the weight of cane in single plots within the larger plots, ranging in size from .023 to .069 acre. The juice data, that is the quality ratio values, are mill reports.

The tonnages of the Kailua crops are based on the weight of cane from three sample lines within each plot. The juice datum of the Kailua plant crop is a mill record, while the juice data of the two Kailua ratoon crops are based on juice extracted by a small three-roller mill together with the chemical analysis of sucrose in the cane.

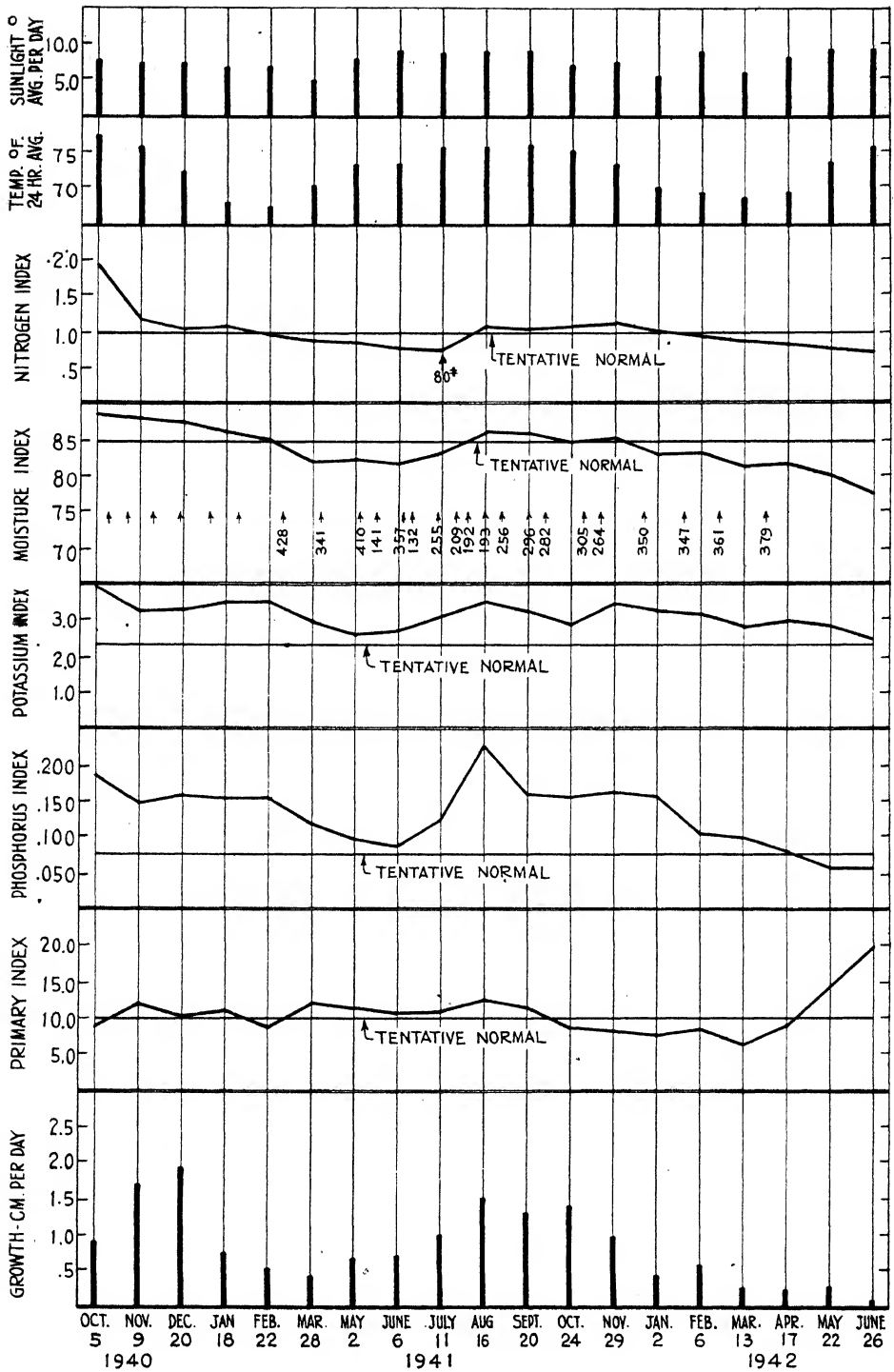


FIG.2. CROP LOG FOR WAIPIO PLOT RB.

Waipio Plot RB:

Plot RB (Fig. 2) was begun August 28, 1940, following the harvest of the plant crop. The plant crop had suffered a good deal from breakage and also from rat damage, but what was there at harvest had been well matured. The phosphorus index of the plant crop had been high, hence no phosphate was applied to the ratoon crop. As in RA, the potassium index was moderately high and until more specific information was available, we decided against taking the risk of leaving it off. Two hundred pounds of potash as muriate of potash are applied together with one hundred pounds of nitrogen as sulphate of ammonia. Again, this application of nitrogen may seem excessive, but later events showed it to be wise. The soil is warm at this season, the stools coming from the plant crop had a good supply of carbohydrate material, and with two to three months of hot weather ahead, the crop can be forced vigorously. There is evidence in these crops that making a heavy initial application under such conditions contributes to the rapid establishment of a dense stand of ratoon shoots.

October 5: Growth was rapid after only five weeks from the plant crop harvest. The primary index is normal, moisture and nitrogen are very high. No change in management.

November 9: The primary index is up, moisture is still very high and nitrogen is dropping. Growth is heavy. No change in management.

December 20: All indices normal or slightly above. (It is interesting to note the very heavy growth that was made so late in the year, somewhat more than two feet since November 9. If we can get such growth by forcing the very young crop as it enters winter, it is that much gained.)

January 18: All indices normal, growth is sharply reduced, but still very good for the season.

February 22: Primary index is dropping, but so early in the cycle, this is not alarming. Moisture is dropping, but despite a light irrigation program, it is still normal. We could shorten the interval between irrigations, but there isn't much to lose at this season of the year. Besides, hardening is taking place.

March 28: The primary index is up. Moisture has dropped sharply and the nitrogen curve is continuing downward. Hardening continues. The crop is in excellent shape and still in very good color, although we could risk the crop going down now, we decide to let it go for another period.

May 2: The primary index is still above normal. Moisture is still low and nitrogen continuous downward. More frequent irrigations are decided upon, but because of some difficulty with water, only one irrigation was made before the end of the period.

June 6: The primary index is above normal. Moisture is actually lower than on May 2. Nitrogen is still dropping. Now we are being confronted with a serious situation. Because of the fact that irrigations were not satisfactory, we cannot be certain that nitrogen is really becoming limiting. If we apply nitrogen without knowing that the plant has exhausted its supply, we are likely to add excessive amounts. Hence, we delay applying nitrogen but we hasten irrigations.

July 11: The primary index is about the same, moisture is rising satisfactorily, but the nitrogen index is still dropping. Hence we can now be reasonably certain

that the crop has used up most of the original 100 pounds. With warm weather upon us, we determine the amount of nitrogen to add on the basis of the sunlight experienced in relation to the contemplated sunlight. This works out to 80 pounds. The nitrogen is applied in the irrigation water and the irrigation interval is shortened to 200 day-degrees.

August 16: Moisture and nitrogen have both risen sharply but so has the primary index. (Here we note that immediately after raising the nitrogen level of the plant, photosynthetic production is stimulated more rapidly than growth.) With moisture above normal, we decide to lengthen the irrigation interval by 50 day-degrees. (Caution: this lengthening process must not be severe, for blossoming might be induced.)

September 20: The primary index is down but is still above normal. Moisture and nitrogen are both high. Hence we extend the irrigation interval another 50 day-degrees, to 300. (It may be noted that the hardening period in spring had been very successful. The cane in lodging had gone down uniformly with no dead cane apparent and with no tendency for the tops to be bunched in large piles, leaving "holes" in the field. There is no evidence of suckering.)

October 24: The primary index is below normal, but there is no need for alarm. The crop is to be harvested about July 1 and hence with a well-hardened crop, we may as well add as many tons as possible at this stage. Moisture and nitrogen are both normal. No change in management.

November 29: The primary index is still dropping. Moisture and nitrogen have risen. We may as well save in costs by lengthening the irrigation interval to 350 day-degrees.

January 2: The primary index is below normal, moisture and nitrogen have dropped. No change in management.

February 6: The primary index is still slightly below normal. No change in management.

March 13: The primary index is down (due largely to very cold, cloudy weather). However, we need not be alarmed for we know that May and June are good maturing months, but to be on the safe side, we lengthen the irrigation interval to 375 day-degrees.

April 17: The primary index is rising, but the crop is now about two and a half months from harvest and with the moisture index tending to rise, we stop irrigations altogether.

May 22: The primary index has risen sharply. Moisture and nitrogen are both dropping.

June 26: The primary index is very high, moisture very low. The crop is ready for harvest.

The yield was 115.6 tons of cane with a Q.R. of 7.1, or 16.28 tons of sugar per acre. At harvest the cane was in excellent condition. This appears to have been a very successful crop. Twenty-eight rounds of irrigation, 180 pounds of nitrogen and 200 pounds of potash were applied. The potash probably was unnecessary. Both the phosphorus and potassium indices are well above normal.

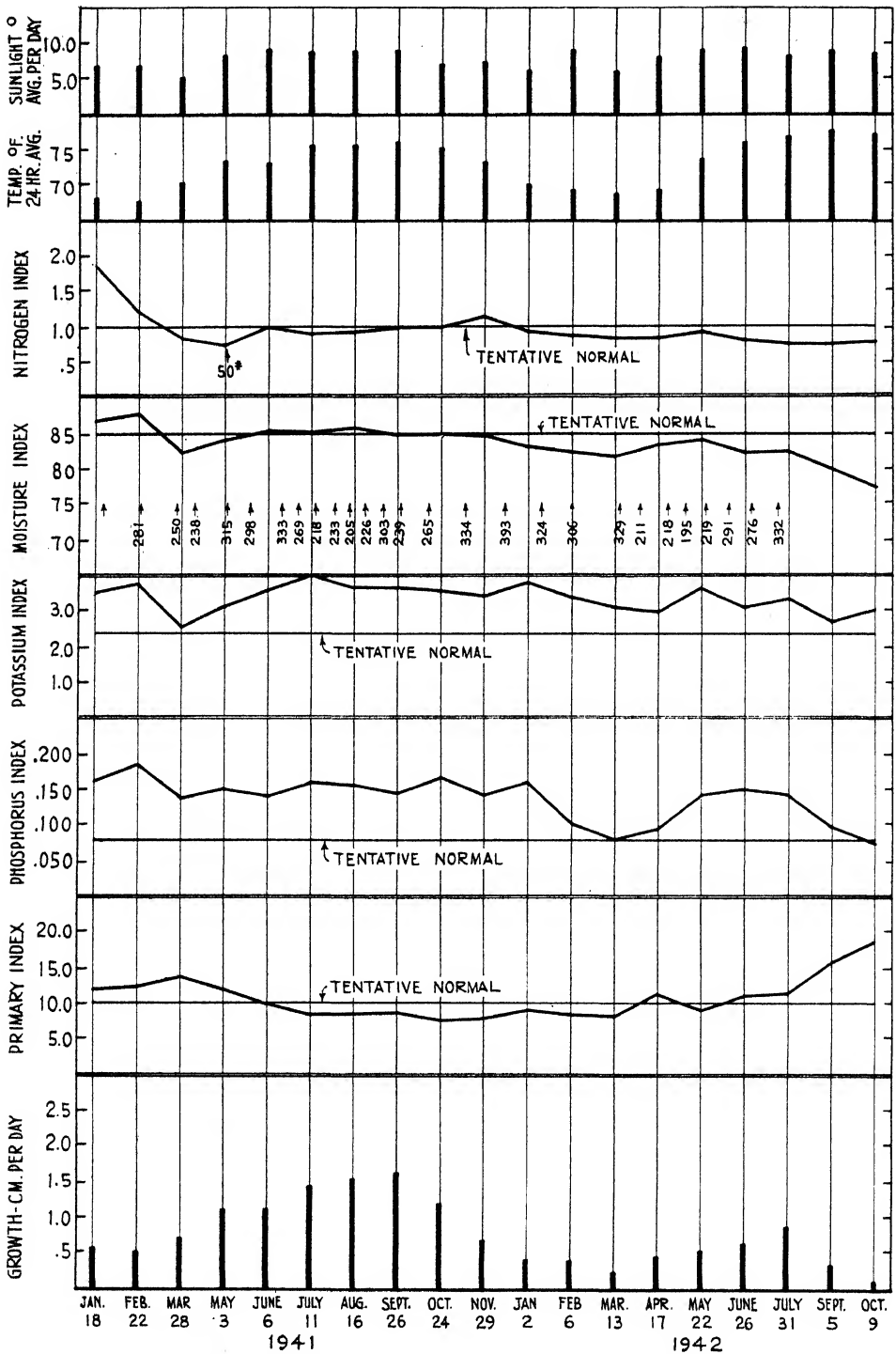


FIG.3. CROP LOG FOR WAIPIO PLOT RC.

Waipio Plot RC:

Waipio Plot RC (Fig. 3) was begun December 1, 1940. The plant crop which preceded it was not a good crop. The yield was 117 tons but the Q.R. was 9.0, yielding 13 tons of sugar. Several reasons can be given for the failure: The crop was badly broken because the mid-crop hardening had not been employed. Rat damage was heavy, and the nitrogen applied was excessive. All of these notes are very important to the fertilizer program of the ratoon, a fact not appreciated until a few months later, and which will be apparent presently. Plot RC was fertilized as were RA and RB—200 pounds of potash and 100 pounds of nitrogen.

January 18: The primary index is high, but both nitrogen and moisture are very high.

February 22: The primary index is rising, moisture is still very high but the nitrogen is dropping very rapidly, a condition usually associated with rapid growth, but the growth is slow. Something is developing here which has not before been encountered.

March 28: The primary index is higher still, moisture is very low for so young a crop and the nitrogen is still dropping. Now, ordinarily this would mean a deficiency of nitrogen since the irrigation interval was being maintained, but only four months before, the crop had received a hundred pounds. What has become of it? In the thought that perhaps something had gone wrong with the irrigation program, a very heavy application of water was made at once.

May 3: The primary index had dropped somewhat, moisture was rising but nitrogen now had reached a critical low. By this time the crop was yellowing badly and obviously something had to be done. The problem still is, what became of the nitrogen that was put on in December? At any rate, the plant was not getting it. To renew the full application would be dangerous, for if the plant finally found the nitrogen previously applied, it would have a great deal too much. So a conservative application of 50 pounds was put on at once.

June 6: The crop has become greener. The primary index is normal. Moisture and nitrogen are again normal. But now other problems confront us. At the rate the crop is now growing, it will begin to lodge in September and October. If we want to harden the crop before it goes down, we shall have to do it before then. At this time the crop is not far enough along to make hardening worthwhile. If we wait until August for hardening, we are likely to set off blossoming. But to help us out of this quandary is the realization that the crop has suffered a good deal and is considerably behind its growth schedule. Possibly even though we push the crop, it may not go down until later, or because it is light, if it did go down, it would not be damaged seriously. So for the time being, we shorten the irrigation interval.

July 11: The primary index is down. Moisture is normal but nitrogen is somewhat low. If it were not for the fact that the original hundred pounds had been put on and very likely was still somewhere in the soil, we would now make a second fifty-pound application. (It would have been much wiser apparently to have used a greatly reduced initial application—especially when the harvested crop had a poor quality ratio and especially during the winter.) But the nitrogen level is not dangerously low. We decide to be conservative and make no further application of nitrogen but we shorten the irrigation interval.

August 16: The primary index is down. Moisture is somewhat above normal, nitrogen is dropping, but the crop is in good color. Growth is fair. We can now begin to lengthen the irrigation interval.

September 26: The primary index remains steady. Moisture is normal and nitrogen is rising. By now it is becoming apparent that the crop is using the nitrogen originally applied.

October 24: The primary index is low, moisture normal, nitrogen steady. The danger of inducing blossoming is now past. We can save some water and at the same time harden the crop which by now is lodged, but there has been damage. The irrigation interval is lengthened.

November 29: The primary index has risen slightly, moisture is normal, but nitrogen has risen. This rise is the result of continued absorption by the roots associated with reduced growth at this late season. That original poundage of nitrogen has not escaped and is being used by the crop. The crop is now about a year old. If we are to add more nitrogen it will have to be done soon. But there are several considerations which have to be weighed. In the first place, the nitrogen level is higher now than it has been since February 22. Secondly, because of the difficulties which obtained early in the cycle, the crop is only fair. Thirdly, to round out a difficult complex, the crop is to be harvested next October—a period which is difficult for maturing a crop. If we apply nitrogen, we may increase the tonnage, but the likelihood of having very poor quality is greatly enhanced. If we are to have a well-matured crop, so that the next ratoon will have a better chance of success than the present crop, all signs tell us not to apply nitrogen at all. So far a total of 150 pounds has been applied. At any rate we can wait another month before finally deciding.

January 2: The primary index has risen slightly, both moisture and nitrogen have dropped. This month is about our last chance to apply nitrogen. We decide against it for these reasons: the drop in moisture is caused by the long irrigation interval, but even so the primary index is low, the color of the crop is good, hence the crop is making sugar, and time is not being lost. Also, the next few months are not good growing months. If we applied nitrogen now it would be there to plague us at harvest time.

February 6: The primary index is again dropping, moisture and nitrogen are also dropping. We could shorten the interval between irrigations but with the low temperatures and low light intensity we can continue with the same schedule, save a little on costs, and not lose much in the way of growth.

March 13: The primary index is still dropping, moisture and nitrogen are getting dangerously low. The crop is beginning to suffer, so the irrigation interval is drastically reduced, from 325 day-degrees to 200. This increased moisture should reverse the trends in both nitrogen and moisture and enable the crop to add a few tons to its growth.

April 17: The primary index has risen sharply. Moisture and nitrogen are both rising. The rise in the primary index again shows the stimulation of photosynthetic activity before growth activity.

May 22: The primary index is below normal again, moisture and nitrogen are returning nearly to normal. Because the primary index has dropped, we lengthen the irrigation to 275 day-degrees.

June 26: The primary index is up now. Harvest is about three months away. At this season, drying conditions are becoming excellent. Should we cut irrigation? If we do, the crop being very low in nitrogen, will dry out too rapidly. Hence we lengthen the irrigation interval to 325 day-degrees.

July 31: The primary index has risen only slightly. Moisture is moderately low and nitrogen very low. Harvest is two months away. Irrigations are discontinued.

September 5: The primary index has risen sharply. Moisture has dropped, nitrogen has been very low. Actually, had it been possible to harvest the crop now, it was ready for it, but the machinery was not available.

October 9: The primary index has continued to rise, moisture is very low and nitrogen is also very low.

At this time the crop is harvested. To continue with the misfortunes experienced all the way through the cycle, the grab-harvester develops a broken clutch and the burned and cut crop remains in the field three days before it is milled. But despite all these adversities, the crop weighed 89.2 tons and had a quality ratio of 7.3. Thus the yield was 12.2 tons of sugar per acre—a yield not to be compared with the previous two crops, but not to be regarded as a total failure.

Because the crop was so difficult, perhaps it is worthwhile now to enumerate the mistakes that were made, and to suggest general principles of handling.

(1) Although Plots RA and RB used successfully the heavy initial application of nitrogen, Plot RC did not. Two factors suggest themselves as casual: (a) The plant crop preceding RC had a very poor quality ratio (9.0) whereas, RA and RB were preceded by well-matured crops (7.2 and 7.5). This fact suggests that the stools and roots of the first two crops were nearly dormant and adequately supplied with carbohydrates. They were, thus, in a position to respond to the heavy application. Plot RC, however, was preceded by a crop still growing at the time of harvest. The stools and roots had not reached dormancy, and were low in available food, hence the heavy application of nitrogen was an error. (b) Plots RA and RB were begun at a time when the soil temperature was rising, hence the nitrogen applied was readily absorbed. Plot RC, on the other hand, was started on December 1, a time when soil temperatures are dropping. Under these circumstances, appreciated as an afterthought, it would have been much wiser to delay the initial application to a later date, or to have greatly reduced it.

(2) On May 3, 1941 when the nitrogen was very low, and had more of a field area been available, it would have been wise to institute an experiment. One block would have received no nitrogen at that time, another would have received the 50 pounds as applied, and another would have received 100 pounds. By following each of the three blocks, we would have obtained much valuable information, useful in later crops.

(3) A similar crisis occurred on November 29 and Jan. 2 (1941–42). Should we have applied more nitrogen on one of these dates? To answer this question, field blocks could again have been set up to answer the question. However, in this experiment only a small plot was available and hence the more apparent, conservative program was followed.

(4) Although specific data are lacking, it appears that losses which are suffered during the early months of the crop cycle are rather directly reflected in the final

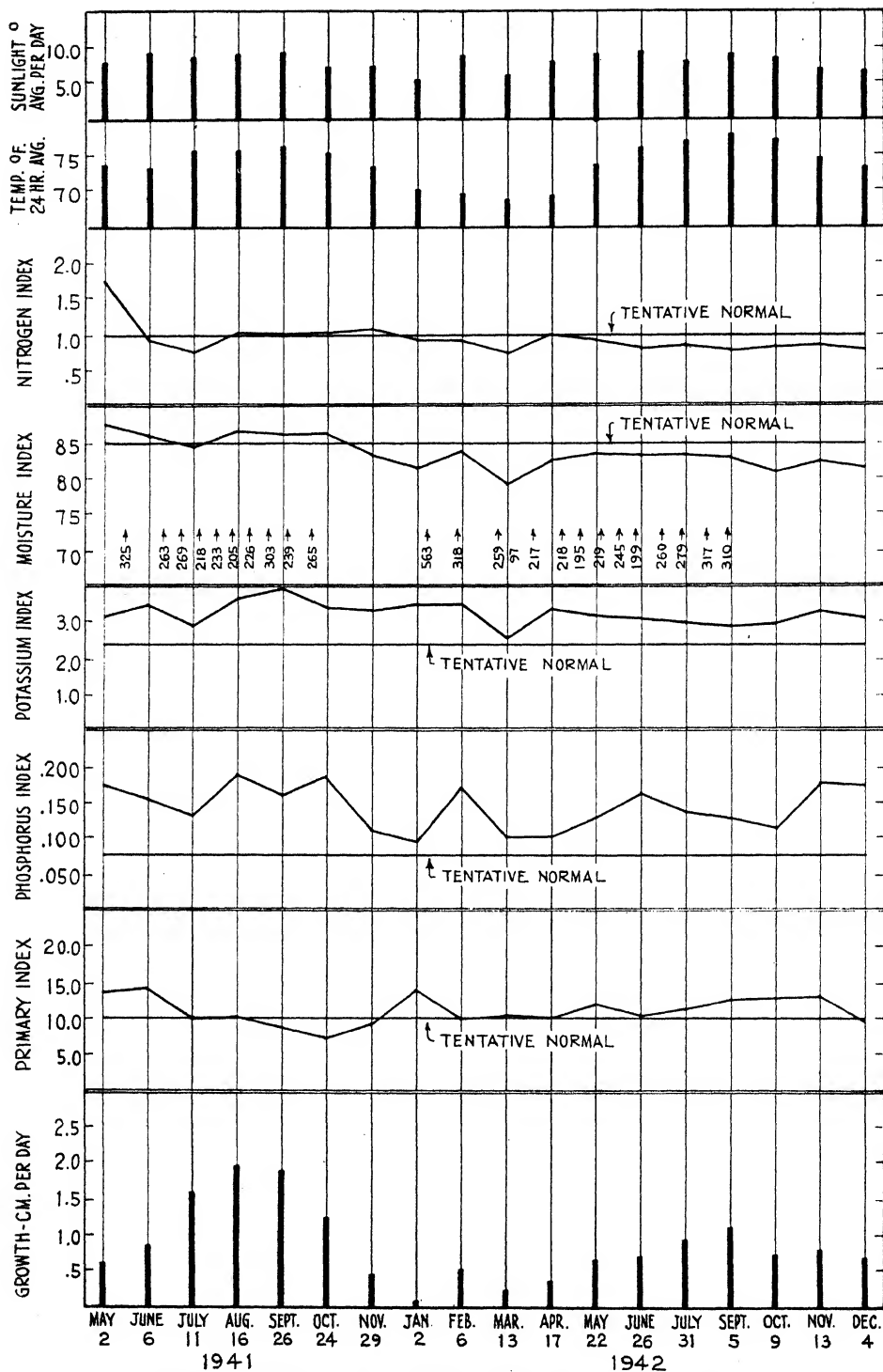


FIG.4. CROP LOG FOR WAIPIO PLOT RD.

crop. It seems likely that attempts during later periods to overcome the initial losses are likely to be defeated by poor quality, resulting from the excess nitrogen.

(5) Finally, a tentative observation may be made for which the crop logs of plots RA, RB, and RC are offered as evidence. If the application of nitrogen to a crop is made when the crop is in a physiological position to absorb all of it or nearly so, the regulation of that crop is simplified enormously over the situation where the nitrogen fertilizer is applied either in great excess or at a time when the absorption capacity of the roots is limited by low temperatures or by paucity of reserve carbohydrates. In the latter instances the fertilizer is distributed through the soil and away from the roots. Although this nitrogen under the conditions of the crops described above was not lost, it was available to the crop at a slow rate, probably only as the roots grew into the soil areas containing the fertilizer. When the fertilizer is applied so that the plant absorbs nearly all of it, the management of the crop is greatly simplified.

Waipio Plot RD:

The crop (Fig. 4) preceding this ratoon was a fair crop, harvested March 3, 1941, with a quality ratio of 7.9. The fertilizers applied at that time were the same as for the previous plots, namely, 100 pounds of nitrogen as sulfate of ammonia, and 200 pounds of potash as muriate of potash. No phosphate was added. Of course, we know now that the heavy application of nitrogen was not wise, but the difficulties experienced with Plot RC were not yet apparent. However, the ratoon of Plot RD followed a better crop than that preceding RC and also we are nearer favorable soil temperatures.

May 2: The primary index is high. Moisture and nitrogen are both high. The nights are cold, but the days are bright and warm.

June 6: The primary index is high, and is rising, moisture and nitrogen are dropping, and the nitrogen curve is doing the same thing it did in RC. We shorten the irrigation interval to 250 day-degrees.

July 11: The primary index has dropped, but moisture and nitrogen are also dropping, the latter to a dangerous low. The young crop is losing color. Exactly the same thing has happened as with RC. This time we won't wait any longer for the summer is upon us. We apply 50 pounds of nitrogen and reduce the irrigation interval to 225 day-degrees.

August 16: Now, the crop has lost its yellow-brown color. The primary index is normal. Moisture is high and nitrogen is normal. No change in management.

September 26: The primary index is below normal, moisture and nitrogen are steady. The irrigation interval is lengthened to reduce costs.

October 24: The primary index is low, moisture and nitrogen are steady. The crop is coming along beautifully. The stand is very heavy and the plants are becoming very tall. We are beyond the danger of inducing blossoming and, to consolidate our gains to date, we undertake to harden the crop during a time when we would not lose much in the way of growth anyway. We stop irrigations.

November 29: The primary index has risen, moisture is down somewhat, nitrogen has risen. The plants are still very erect. Hardening has begun, but has not progressed very far. We continue the hardening program.

January 2: The primary index has risen sharply. Moisture is low. Irrigation is resumed.

February 6: The primary index is normal. Moisture is rising and nitrogen is steady. No change in management.

March 13: Although the primary index is normal, the serious drop in moisture and nitrogen requires attention. This drop was apparently caused by a period of high winds and low humidities extending from about February 10 to March 6, but we have reached the period where the final application of nitrogen has to be made. The situation is complicated by the fact that the temporary drought has lowered the nitrogen level of the plant below what it would be at normal hydration. Hence in making our calculations, we go to the preceding analysis, February 6. Here we note the nitrogen level at 0.93 per cent. If we consider 1.00 per cent as normal and .75 as the absolute bottom of the range we determine the proportion .93 is of this range or 18/25. We calculate the amount of sunlight experienced by the crop, also that contemplated. Since 150 pounds carried the crop through the energy available, and at the same time was within .07 per cent point of normal, then 7/25 of the proportional amount obtained for the anticipated amount should carry the crop the remainder of the cycle. This amount is 30 pounds, which is then applied in the irrigation water. Also, in order to get the crop going as soon as possible, the irrigation interval is shortened to 200 day-degrees. (It should be remembered that the nitrogen applied is conservative because the crop has to be matured in December, not a good time.)

April 17: The primary index is normal. Moisture and nitrogen both have improved. No change in management.

May 22: The primary index has risen but moisture is nearing normal, nitrogen is dropping somewhat.

June 26: The primary index has dropped again. With harvest six months away, we do not want the index to continue this drop, so we lengthen the irrigation interval to 275 day-degrees.

July 31: The primary index is rising, growth is fair. Moisture and nitrogen are below normal. Harvest is 5 months away. No change in management.

September 5: The primary index continues to rise satisfactorily but we are approaching another critical time. With harvest about three and a half months away, weather for maturing the crop is not likely to be very good. Also, the chances of fall rains compel us to start the crop drying. The trend for the primary index during September and October is generally downward, and with the crop so near harvest we must hold the primary index up, unless quality is to suffer. If we can severely check the crop with its low nitrogen level, rains are not likely to affect seriously the quality. We are in danger of setting off blossom, but the drying may be sufficiently intense to prevent that. Hence we discontinue irrigations.

October 9: The primary index has risen very slightly, moisture has dropped, nitrogen has risen slightly. No change in management.

November 13: The primary index is steady, moisture has risen somewhat. Thus, despite the rains (some of them very heavy) since the last part of October, and the warm weather, the crop remains moderately low in moisture. Had excessive nitrogen applications been made, such nitrogen, whether a reserve in the plant or in the soil, would have caused a rapid rise in moisture.

December 4: Rains continued throughout the period. Cloudy weather prevailed. The primary index has dropped but because of the low nitrogen levels, moisture remains low. The crop is harvested. The preharvest burn was poor because of the rains, but despite a 10 per cent weight deduction, the yield was 119.4 tons of cane, the quality ratio, 8.2, and the sugar yield 14.6 tons. The crop received 28 rounds of irrigation, 180 pounds of nitrogen and 200 pounds of K_2O as muriate of potash.

Thus it is apparent that by proper control of crops, it is possible to get reasonably good quality crops even during the worst part of the year. Had nitrogen been applied in excess of the needs of either Plot RC or RD, the tonnage would have been larger (most of the increase being pure water) increasing the cost of harvesting, and the quality would have been poorer. There might even have been less sugar.

CROPS AT KAILUA

The field on which the crops were grown at Kailua is near the base of the mountains. It is in an area of low light intensity, being generally cloudy. Late afternoon shadows from the mountains further reduce the total amount of energy available to the crop. The field is without irrigation facilities. During the crop cycles, there was usually enough rain to maintain growth. Unfortunately, in this area water is likely to be limiting in the late spring and summer, at the time when most growth should be made. When moisture is abundant, during the winter, it is usually too cold for growth. Under such circumstances there isn't much which can be done in managing a crop. But there are some things which had been learned in the plant-crop cycles which were applied. Although the results are far from satisfying, they are presented with the idea that they represent conditions very nearly at their worst and that even under such conditions certain controls are possible. Although the plant crops were fair, in general the ratoon crops, except for RC, started out in an excellent manner. During 1941, however, there was a marked shortage of water, which not only interfered with growth of the crop but seemed to encourage heavy rat invasions. The field used for these studies is completely surrounded by pasture and wasteland and control of the rats under such circumstances was most difficult. All the ratoon crops were very badly damaged. In some cases solid strips of line 10 to 15 feet long had every plant gnawed away. Obviously, under such circumstances the biotic factor is the most important factor in determining final yield.

From a physiological point of view, as well, rats are a serious menace. For example, when a crop has become established and the fertilizer applications are made, it is with the assumption that the whole crop remains on this field. Thus if the crop averages four plants per linear foot, there are about 36,000 upright stalks per acre. Now, if the nitrogen applied to such a field is 100 pounds, it is presumed that the 36,000 plants will be drawing more or less uniformly on this poundage. However, if half the plants are chewed off, or damaged, then there is an excess of the nitrogen for the remaining plants on the field. As the distal portion of the chewed-off stalks begins to disintegrate its nitrogen is leached from it to add to the general excess. Under such conditions we may expect heavy suckering and a serious reduction of quality. Of course the stalks which are partially chewed, even though they continue to grow, will be subject to red rot extending for varying distances up the cane.

When the earlier paper in this series was published, there was some criticism of it on the grounds that the Kailua crops were suffering from phosphorus deficiency.

Even though abundant evidence was offered that such was not the case, yet in deference to the criticism when the ratoon crops were started an especially heavy application of phosphate was applied (400 pounds of P_2O_5) as reverted phosphate. After harvesting the plant crop, a deep furrow was made within one foot of the line of plants, the phosphate applied, and then the furrow was filled in. The object of all this was to place the phosphate where the new roots would reach it. Further, reverted phosphate was used with the idea that the roots would be in the area before all of the phosphate was fixed by the soil. The phosphate index of the plants showed that the phosphate level within the plant was raised considerably, but there is nothing to indicate that the nutrition of the plant was improved by it.

The calcium index of the Kailua-grown plant crops was also at a level very much lower than the corresponding crops at Waipio. Again as an insurance against the possibility that calcium may be limiting, despite much evidence that calcium is never limiting in Hawaiian soils, calcium was applied with nitrogen as calcium nitrate. Again, the levels in the plant were materially raised. The common belief that applying lime to cane reduces its quality could not be verified here. Despite the increase in calcium levels within the plant, quality was decidedly better in the ratoons, but probably not because of the added calcium. If liming causes declining quality, the influencing factor must be other than the calcium composition of the cane. These data will be presented in detail later. Two hundred pounds of potash were applied as muriate.

One final factor needs be presented before proceeding with the crop logs for the Kailua-grown ratoons. In areas where the moisture available to a crop is supplied by rain, there are no means of controlling the supply. However, in this study there have been repeated instances of high correlations between the moisture content of the plant and its nitrogen level. These studies have in many instances shown that where nitrogen is available to the crop, if moisture becomes limiting, the nitrogen content of plant drops. Conversely, where nitrogen is limiting, even though moisture is available, the moisture content of the plant is at a low level. This fact seems to offer considerable hope to those plantations in heavy rainfall areas. If by impressing a physiological drought on the crop, we may obtain reasonably good quality we should not only reduce fertilizer costs, milling costs, but actually produce more sugar. Because of this situation, the nitrogen applied to the ratoons was all applied at once as 100 pounds of nitrogen as calcium nitrate. It is apparent now that it would have been better to break up this quantity, but the logs as presented later do verify the principle of moisture control through nitrogen application.

Kailua Plot RA:

The plant crop was harvested on May 28, 1940. The quality ratio was 9.0. The ratoon crop was immediately fertilized. Response was good despite low rainfall. The stand was dense.

August 3 (Fig. 5) : The primary index is high, nitrogen is moderate for so young a crop, and moisture is very low. But the summer drought seems to be about broken.

September 7: The primary index is now where it should be. Moisture is normal and nitrogen is high considering the growth that is being made.

October 6: The primary index is low, moisture and nitrogen are both high. The crop is doing very well. Evidences of moderate blossoming are apparent.

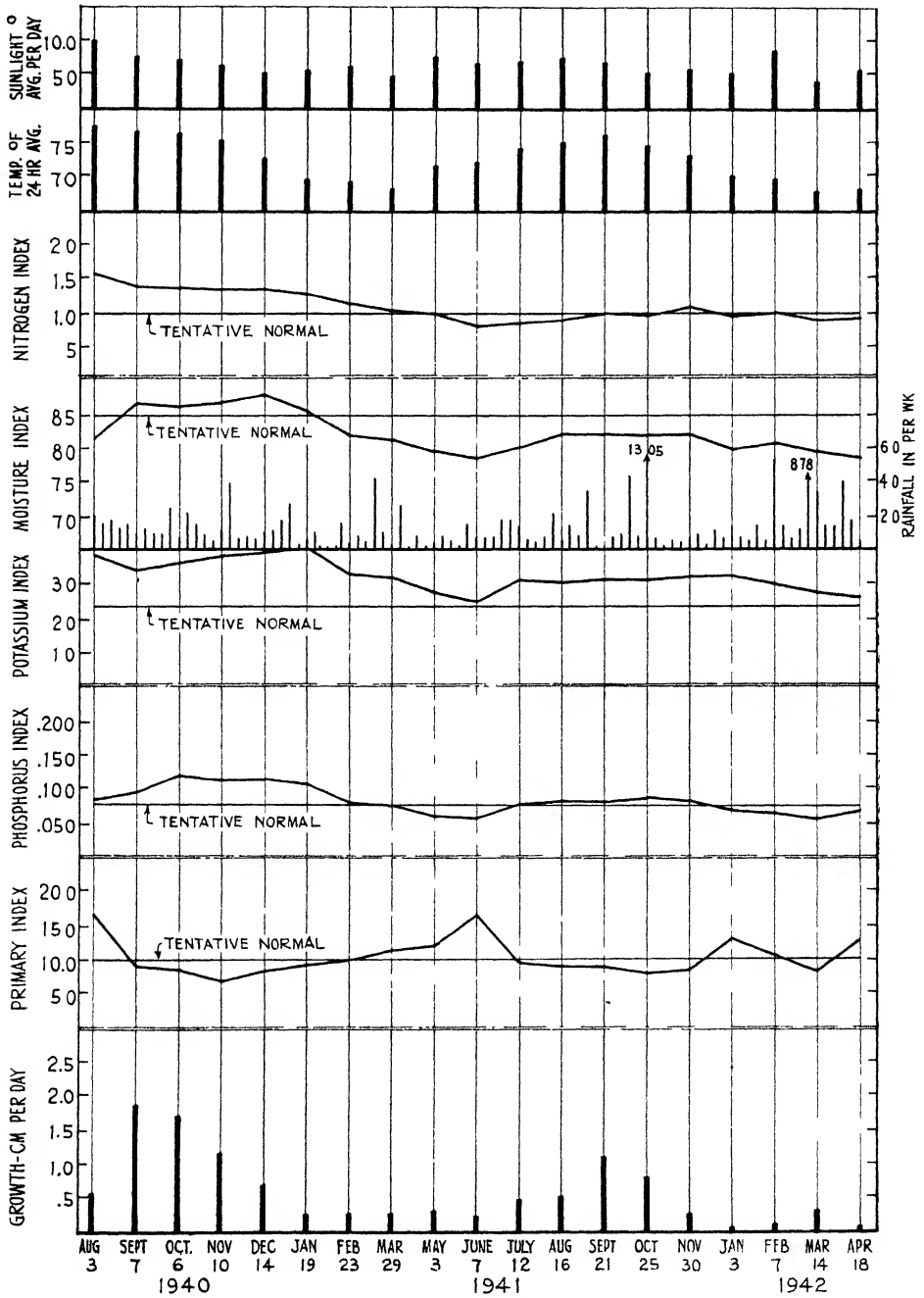


FIG 5 CROP LOG FOR KAILUA PLOT RA.

November 10: The primary index is very low, moisture and nitrogen are high. Thus, so far we have produced a most promising young crop. Blossoming is moderate and the plants have attained a good height.

December 14: The primary index has risen though it is still low, with moisture and nitrogen still high.

January 19: Primary index is normal, moisture is normal although lower than previously, nitrogen is high.

February 23: Primary index is rising, moisture has begun to drop followed by nitrogen. During this period, there were three successive weeks with only traces of rainfall. Colder weather is also playing a part.

March 29: The primary index is still rising, moisture and nitrogen are both dropping. Despite one good week of rain, the crop is experiencing a shortage of water.

May 3: The same trends continue. Moisture is becoming very low. The plants are now beginning to go down, and many of them, probably because of the dry, hard soil, are breaking off completely and are dying. Rat damage on leaning stalks is becoming very severe, despite rat baiting and poisoning.

June 7: The drought is now extremely severe. The primary index is very high, nitrogen very low, and moisture extremely low. The breakage of plants by lodging and rat damage is a most discouraging sight. What was once such a promising crop, is now a mess.

July 12: Several fair rains, combined with warm weather, have broken the drought. The primary index is normal. Moisture is rising followed slowly by nitrogen.

August 16: Despite barely adequate rains, the primary index has dropped somewhat, moisture has risen and nitrogen, which apparently is not abundant in the soil any more, is rising slowly.

September 21: All indices are steady with a slight rise in nitrogen. But now with excellent temperatures for rapid growth, and seemingly enough rain, the moisture curve remains considerably below normal. In the plant crop where more nitrogen was applied, under similar conditions, there was a very rapid rise in both nitrogen and moisture to points above normal. In this ratoon, however, nitrogen is becoming limiting and is affecting the moisture content of the plant.

October 25: The same situation continues despite abundant rainfall and favorable temperatures.

November 30: Despite a drenching rain, the moisture content remains low.

January 3: Colder weather, somewhat reduced rains, although not a drought, raises the primary index, drops moisture and nitrogen. Harvest is three months away.

February 7: The primary index is down, moisture has risen somewhat but is still low.

March 14: A very cold, cloudy period. The primary index is down, but despite considerable rainfall, the moisture index is lower as is nitrogen.

April 18: Following a period of improved temperature and with abundant soil moisture, moisture is lower than that at Waipio (see Fig. 1). Nitrogen is below normal, though not materially so. The primary index is up.

The crop is harvested and yields 42.9 tons of cane with a quality ratio of 7.39, or

5.81 tons of sugar. Now, to be sure, this is not a satisfying yield, but it does seem clear that even though crops in the high rainfall area are subjected to rains during the maturing period, they can be made to yield satisfactory quality ratios if nitrogen is limiting. If excessive amounts of the element are applied, they will be there in the soil to plague the grower when harvest comes around.

In this case it seems now that it would be wiser to apply about 50 pounds at the start, and then at the beginning of the second season (in this case, about May 3, 1941) apply the calculated amount. In this way we could probably have had a rapid recovery and better growth during the summer. The yield itself was, of course, greatly reduced because of the extensive rat damage. At harvest about 75 per cent of the stalks were chewed or broken. Obviously where rat infestation is heavy, an important part of the cultural practice should revolve about controlling the menace.

Kailua Plot RB:

The crop which was followed by this ratoon was harvested August 27, 1940. Its quality ratio was 9.5. Plot RB was fertilized at once and with abundant warmth and sufficient rain, it started out with a very heavy stand.

October 6 (Fig. 6): The primary index is low, moisture and nitrogen very high.

November 10: The primary index is still low, although rising, moisture and nitrogen are high.

December 14: Despite colder temperatures, growth is continuing.

January 19: All indices are good.

February 23: Although the rainfall is becoming limiting, this crop at this time has a higher moisture level than RA. (Probably aided by the higher nitrogen level.)

March 29: The primary index is normal, suggesting that the crop is not suffering from the lack of water even though the moisture index is dropping.

May 3: The primary index is now rising, moisture is very low, nitrogen, too, is reflecting the moisture condition.

June 7: The primary index is high, moisture remains low, and nitrogen continues to drop.

July 12: Improved rainfall has lowered the primary index, raised the moisture index. Nitrogen remains steady. (At this point we could have had a speedier recovery of the crop from the drought were we to add some nitrogen. It would have been better to apply less at first and put on a calculated amount at this point.)

August 16: The primary index is normal, moisture is rising slowly, nitrogen is steady.

September 20: The primary index is low, moisture normal and nitrogen nearly so. (In other words there is still enough nitrogen in the soil.) The crop is in excellent condition. It is still erect, very dense, and so far, there has been practically no rat damage, despite the heavy infestation in the adjoining Plot RA. However, the crop (RB) is becoming topheavy and is likely to go down very soon.

October 25: With the very heavy rainstorm, the crop has gone down, but probably because of the hardening induced by the drought, there appears to be very little breakage. The primary index is down, moisture has dropped, as has nitrogen.

November 30: Despite rat baiting and poisoning, rat damage is heavy. The primary index is still low. Nitrogen is normal, moisture is steady.

January 3: The rat damage becomes extremely severe.

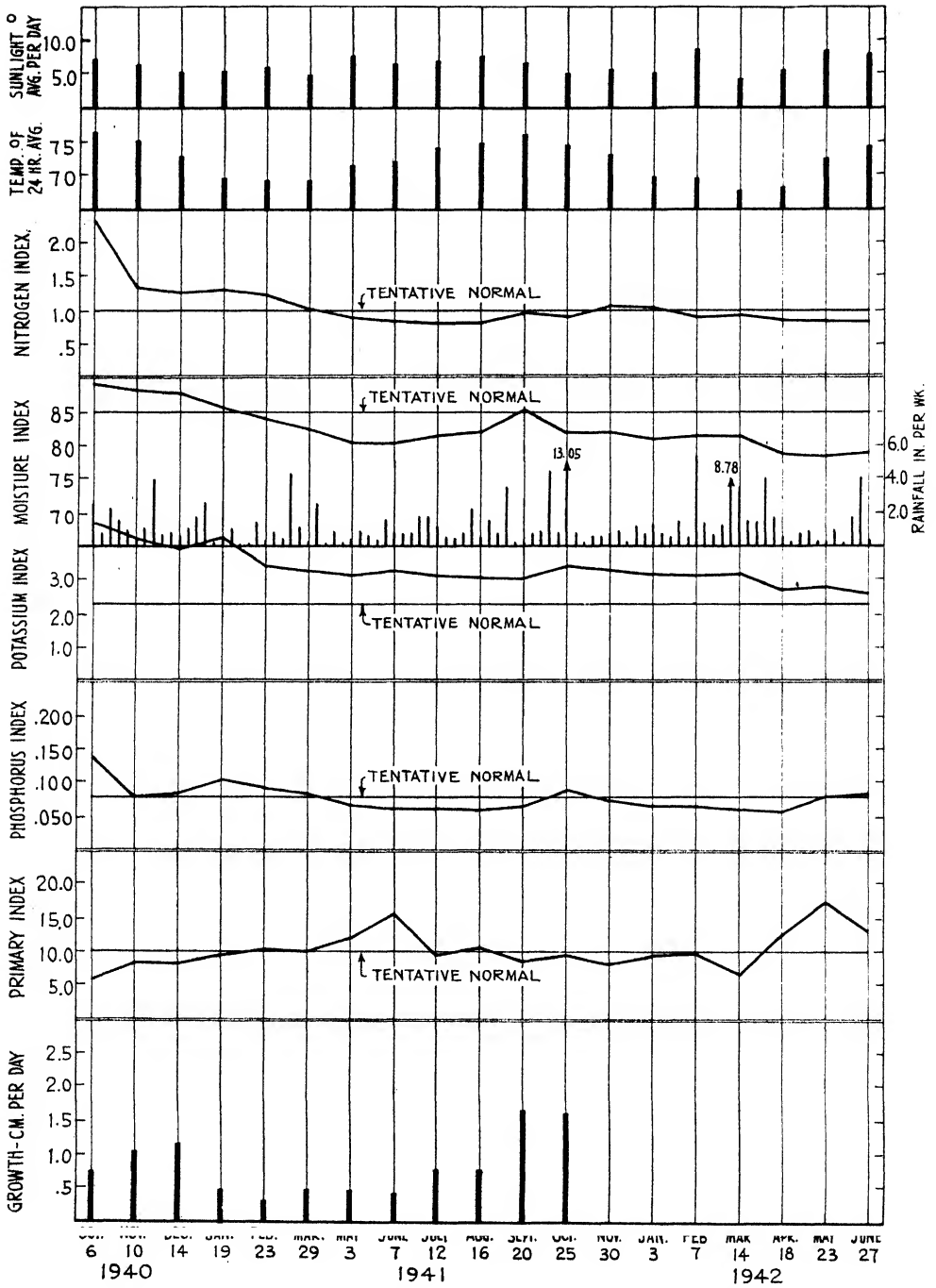


FIG. 6. CROP LOG FOR KAILUA PLOT RB.

February 7: The primary index is normal, moisture continues low and nitrogen is dropping.

March 14: After a very cold, cloudy period the primary index has dropped, even though nitrogen and moisture are both below normal.

April 18: Here we begin to get the real effects of the developing nitrogen deficiency. The primary index is rising and moisture and nitrogen both drop, despite heavy rains.

May 23: The same trends continue.

June 27: With rising temperatures, the primary index drops, but is still high, moisture and nitrogen are both low.

The crop is harvested and yields 55.2 tons of cane with a quality ratio of 6.99, thus yielding 7.9 tons of sugar, a crop which can be regarded as excellent considering the drought, rat damage, etc.

The relation between nitrogen and moisture is an important one. Kailua Plot RA received 52.70 inches of rain during the first eleven months of its growth and 80.58 inches during the second eleven months. Plot RB received 49.00 inches during the first eleven months and 83.35 during the second eleven months. Despite the heavy rains as harvest approached the moisture levels within the crop remained low, due to the reduced nitrogen levels.

In order to give the reader a case for comparison, the crop log for a higher nitrogen field (KC) is reported. This plot was grown without reference to the curves. The application of nitrogen was made on the basis of what was then accepted practices. At planting an application of Ammo-phos was made to give 200 pounds of P_2O_5 and 44 pounds of nitrogen. Further, 200 pounds of K_2O as muriate of potash were also applied. Two further applications of nitrogen as sulphate of ammonia were made, as indicated on the nitrogen index. For the entire cycle, 160 pounds of nitrogen were applied. Rainfall was adequate, except for two short summer periods. During the first eleven months, 76.56 inches of rain fell and during the second eleven months, 73.16 inches. The field was planted January 28, 1939. The 31-1389 planting material was cut at the Waipio substation, and soaked in warm water ($35^{\circ} C.$) for about 24 hours prior to planting. Germination was excellent, despite cold weather.

Kailua Plot C:

March 24 (Fig. 7): The primary index is low, moisture and nitrogen are very high.

April 21: The primary index is normal, moisture and nitrogen very high. The crop is growing beautifully and is dark green in color.

May 26: The primary index is dropping somewhat, moisture is normal, nitrogen is high. However, the fertilizer schedule calls for a nitrogen application. Fifty-six pounds of nitrogen as ammonium sulphate are applied. It should be obvious that there was no need for this application. The nitrogen index was well above normal. The primary index is low. Moisture is dropping, but this is due to a lighter rainfall.

June 27: The primary index is dropping, as is moisture. Nitrogen is above normal.

July 23: The primary index is steady, nitrogen is high, moisture continues to drop. The drought is now beginning to check growth.

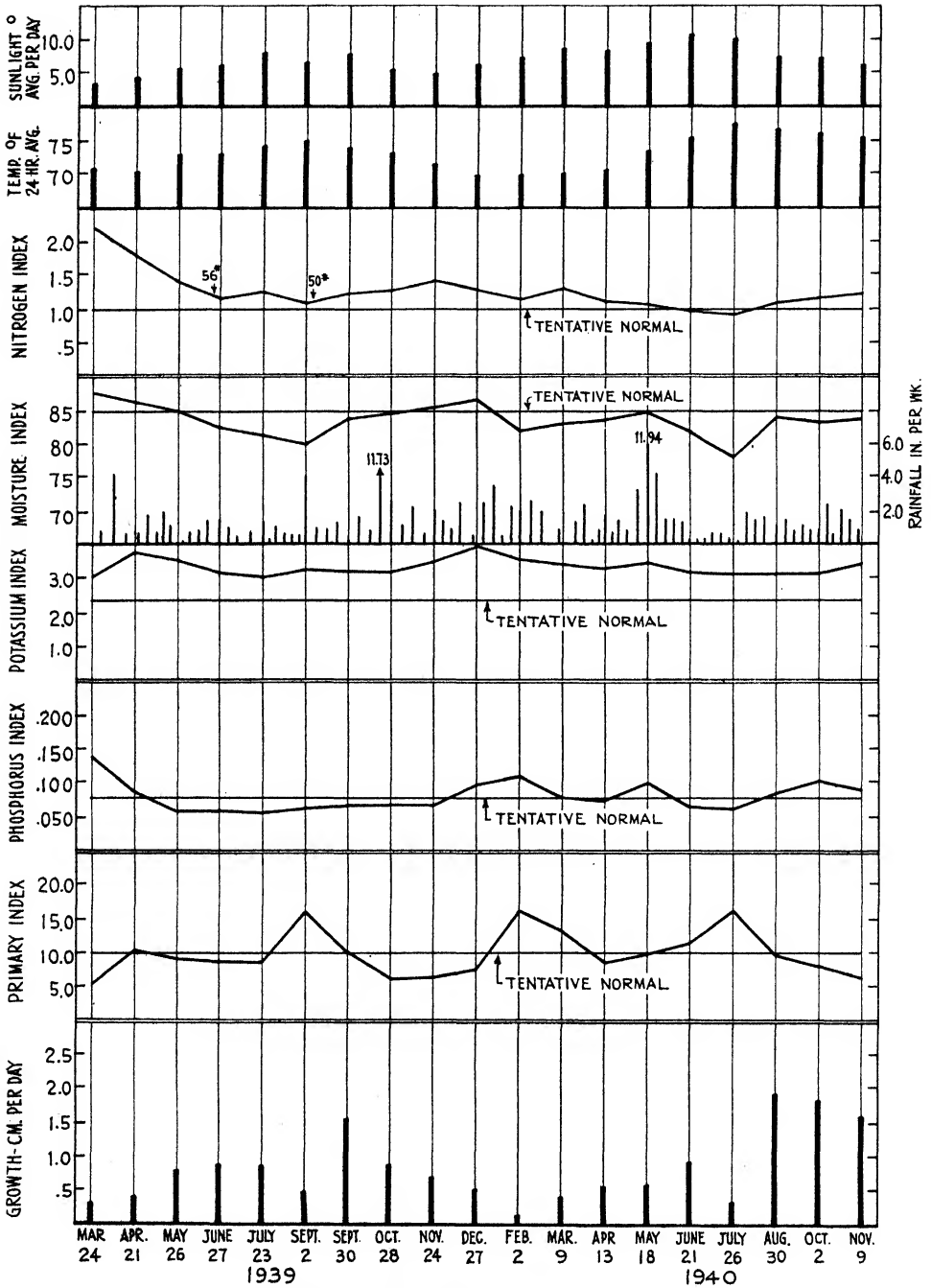


FIG. 7. CROP LOG FOR KAILUA PLOT C.

September 2: The primary index has risen sharply, moisture is low, nitrogen is dropping, but most likely because of the drought. A good four-inch rain fell at this time. The last application of nitrogen was made. (At least one of the two applications of nitrogen was unnecessary.)

September 30: Following the rain, a very rapid recovery was made. Growth is very heavy. The primary index is normal, moisture is nearly normal, nitrogen, despite the heavy growth is rising.

October 28: One very heavy rain and several substantial rains have fallen. The weather is cloudy and hot. The primary index is down. Moisture is normal and nitrogen is very high. (A curious fact asserted itself at this point. Two older plots (6 and 3 months, respectively) and one younger plot (3 months) showed heavy blossoming, but Plot C except for a few blossoms on the edge of the field had no blossoms.)

November 24: The primary index is very low, moisture is high and nitrogen is very high.

December 27: The primary index is low, moisture is high and nitrogen very high.

February 2: Cold weather, and somewhat improved sunshine starts the primary index upward, moisture is down, nitrogen is high.

March 9: The primary index is dropping, moisture and nitrogen are rising.

April 13: All indices are nearly normal.

May 18: All indices are normal. Harvest is about six months away. The crop is dark green and has made excellent growth. There has been very little breakage and practically no rat damage.

June 21: The primary index is rising, moisture is dropping and nitrogen is normal. A drought is beginning.

July 26: The primary index is high, moisture is low and nitrogen is somewhat below normal. Growth was heavily curtailed by the drought. This would be a good time to harvest. However, the schedule calls for harvest about November 1.

August 30: Several good rains, result in a burst of growth. The primary index is down, moisture is nearly normal and nitrogen is rising. With harvest two months away, these curves bode ill.

October 2: The primary index is well below normal, moisture is nearly normal and nitrogen is above normal and is rising with harvest only one month away! Growth is heavy.

November 9: The primary index is very low. Moisture nearly normal and nitrogen very high. The crop is harvested and yields 91 tons of cane with a quality ratio of 12.78 and 7.1 tons of sugar. Compare this yield with KRB, grown under much less favorable circumstances which yielded only 55.2 tons of cane and 7.9 tons of sugar. It should be remembered that rainfall for Plot C was adequate both seasons, whereas rainfall was less than adequate the first season for RA and RB and heavy during the harvest season. To strike a cost comparison between Plot C and RB, 60 per cent more nitrogen was used for Plot C. For each ton of sugar, nearly six additional tons of weight (mostly pure water) had to be harvested and hauled.

The nitrogen curves for Plot KRB and KC may be compared for general levels. The curve for RB was normal or below for the most part after March 29, 1941, while that for C was normal or below on only two occasions, both late in the cycle. The nitrogen index for RB just prior to harvest was below normal and was drop-

ping. That for C was above normal and was rising. It seems very clear that the 160 pounds of nitrogen for Plot C were excessive. Had the additions of nitrogen been made according to the index, at least one of the applications would not have been made. But the excess of nitrogen whether still in the soil or in the plant is really of little moment, since in either case it will be used by the plant when conditions are otherwise favorable. Actually, much of the excess was in the plant. (Cf. nitrogen compositions of cane KC and KRA, pp. 166 and 168, Vol. 46, this journal.)

Two other comparisons may be made. *First:* The potassium index for KC is very high. Had the index been followed at that time, the application made to the ratoon crop would not have been made. *Second:* The phosphorus index for KC was below the tentative normal for the first half of the crop and about normal for the remainder. Even though there is nothing to indicate that phosphate was a deterring factor in growth, a double application of P_2O_5 (400 pounds) was made on the ratoons. During the first half of the ratoon crops (RA and RB) the phosphorus index was normal or above. During that part of the log where moisture was below normal, the phosphorus index dropped. In fact, there appears to be a good correlation between the phosphorus levels of the crop and its moisture content. (KRB, $+ .6476$,** KRA, $.9353$,**.)

GENERAL DISCUSSION

There are two general approaches to the solution of crop problems encountered in the field. One involves the empirical application of various treatments, fertilizers, water, type of planting, etc., upon the crop. Plans are carried out according to schedule and little attention is paid to the crop as it grows. The results of the treatment are determined at harvest time. The second general approach is that reported in this paper. Here certain measurements associated with the welfare of the crop are made at intervals of five weeks. The time of applying fertilizers (except for phosphorus), the quantity of fertilizer, the length of the irrigation intervals, the length of the maturing period, etc., are not worked out before hand but are worked out as the crop progresses, basing the decisions made at any one time on the progress made by the crop as revealed in the crop log. In this way the variations of climate, which are not inconsiderable, are taken into account as the crop progresses. With the establishment of the crop log, a complete record of the crop is available not only for use in guiding the particular crop to its harvest, but serves then as a series of precise measurements which may be used not only for comparison with other fields but over a long series of years with the same field.

The empirical method has a very important place in the crop-log approach. Thus when the time is reached that a decision must be made with reference to a particular practice, for example, the final application of nitrogen in Waipio Plot RC, Fig 3, January 2, 1942, if the grower has not encountered that particular situation before, he can institute a block experiment at that point. The plots are logged and from the subsequent behavior of the curves as well as the final harvest data, he accumulates information which will be useful when similar crises develop in the future.

SUMMARY

1. The elongating cane sheaths are found to be the plant organ within which the total sugar level best serves as the primary index.

2. The primary index is sensitive to variations of sunlight, temperature, growth rate, moisture and perhaps nitrogen, potassium and phosphorus.
 - (a) It is positively correlated with sunlight, temperature, and sometimes nitrogen and phosphorus.
 - (b) It is negatively correlated with growth, moisture, potassium and sometimes nitrogen and phosphorus.
3. The part which the primary index plays is important. Under conditions where growth is below normal for the particular environment, the index rises. Where growth is excessive, the index drops. Because of this behavior, the index may be used as a guide in fertilization, irrigation, and crop management in general.
4. The crop logs of six ratoon crops (four at Waipio and two at Kailua) and one plant crop (Kailua) are presented in detail showing the use of the primary index and the secondary indices in managing the particular crop while it grows.

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